Variability in Development of Oestrogen Target Organs After Induction of Puberty in Adolescents and Young Adults

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BM (Hons), BSc (Hons), MRCOG

This thesis is presented for the degree of

Doctor of Philosophy

University College London

Institute of Women's Health Research Degree

Department of Reproductive Health

THESIS DECLARATION

I, **Elizabeth Burt** confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Several publications have been produced as a result of this work.

Publications

1. Burt E, Davies MC, Yasmin E, Cameron-Pimblett A, Talaulikar V, La Rosa C, Clarke SA, Conway GS. Fertility and pregnancy outcomes in women with Turner syndrome: A single centre experience. Clin Endocrinol (Oxf). 2024 Jul;101(1):51-59

2. Burt, E., & Yasmin, E. (2023). Primary Amenorrhoea and Delayed Puberty (pp. 57-66). In Naomi S. Crouch, Cara E. Williams (Eds.), Paediatric and Adolescent Gynaecology for the MRCOG © Naomi S. Crouch and Cara E. Williams 2023, published by Cambridge University Press

3. Burt E, Ruff C, Yasmin E, Davies M, Cameron-Pimblett A, Butler G, Conway G. Challenges in developing a quantitative method of measuring breast development using 3D imaging: An example of a novel method for use in induced breast development with exogenous oestrogen. Clin Endocrinol (Oxf). 2023 Jan;98(1):68-73.

4. Burt E, Yasmin E, Davies MC, Creighton S, Brain C, Ruff C, Learner HI, Williams L, Cameron-Pimblett A, Talaulikar V, Conway G. Variability of response to early puberty induction demonstrated by transverse uterine

diameter measurement and a novel method of 3D breast imaging. Clin Endocrinol (Oxf). 2022 Jul;97(1):91-99.

5. Burt E, Davies MC, Yasmin E, Cameron-Pimblett A, Mavrelos D, Talaulikar V, Conway GS. Reduced uterine volume after induction of puberty in women with hypogonadism. Clin Endocrinol (Oxf). 2019 Dec;91(6):798-804.

Signed,

Elizabeth Burt

26/10/24

ACKNOWLEDGEMENTS

A PhD is often considered a journey both academically and personally and for this I can vouch. This journey has taken me on an adventure that I never thought possible and has been one that, at times, I never thought would end. It has been filled with immense learning, a new love of academia and has led me to a niche of clinical medicine which I would never have found and explored.

Professor Conway has been the most amazing and patient supervisor. He took me on as an ignorant and very naïve student and has nurtured and encouraged me without hesitation. Without his ongoing support and guidance this work would have simply not been possible. He is a truly remarkable clinician and gentleman. He is inspirational to so many people and I feel very honoured to have had the opportunity to work with him. I don't think any words will ever quite capture my gratitude for everything that he has done to help me.

There are many people who have helped me immensely and who are the most superb mentors and role models. I would like to thank my secondary supervisors Professor Sarah Creighton, Professor Melanie Davies and Miss Ephia Yasmin for all their time and support and overall for being so inspirational. My time in the physics department was one that stemmed from a curious conversation one day to discovering a department that I didn't even know existed. Cliff Ruff is a wonderful colleague and friend. Cliff and Ming have taken me under their wing and helped me to try and understand the maths and physics, again showing me endless patience. Without Cliff going above and beyond with his time and coming up with many innovative ideas, the 3D breast scanning work would never have been possible.

I have had the privilege of working alongside some amazing colleagues including Professor Gary Butler, Dr Caroline Brain, Dr Vikram Talaulikar, Dr Clementina La Rosa, and Dr Sophie Clarke. Dr Antoinette Cameron-Pimblett, a now cherished friend, held my hand with ethics applications showing me the ropes and helping me through the hurdles. Mr Dimitri Mavrelos helped me no end with all the ultrasound work, whilst Professor Hall-Craggs assisted with the MRI work.

I have met some remarkable patients and my research would have not been possible without their commitment and time. I am in the fortunate position of still being able to see many of them in clinic and this continuity of care and rapport is very special to me.

My family have always been my biggest supporters. During this time not only has my academic world flourished but also my family, having had two beautiful children, Amisha and Riyan along the way. They have provided me the unconditional love, smiles and laughter that has kept me grounded and always reminded me about the most precious things in life. My husband, Ash, has been by my side every step of the way giving me the support and freedom to pursue my academic love. My sister, Anna, is my rock, always providing a listening ear when I have faced challenges and giving me the boost I needed to carry on despite the setbacks.

My biggest thanks go to my parents and aunt, who are without doubt my biggest champions, never questioning my capabilities. They are now experts in Paediatric Gynaecology by osmosis and can now quote the optimal uterine size by heart, having proof-read every piece of my work, often multiple times, without question.

Without my mum, dad and aunt my dream of completing a PhD would never have come to fruition.

Whist I am so proud of all the work I have achieved this would never have been possible without the ongoing dedication of the bigger team around me, and for that opportunity I feel incredibly fortunate.

ABSTRACT

INTRODUCTION

Puberty is a fundamental milestone providing female maturity and involving changes within the female body under the influence of oestradiol. Several pathologies may prevent the onset or completion of spontaneous puberty requiring exogenous oestrogen for maturation of oestrogen target organs including uterus, breast and bone. Optimal uterine development and function is a prerequisite to allow an environment necessary for successful embryo implantation and ongoing pregnancy.

The aim of my work is to explore and gain further insight into the variability in oestrogen sensitivity, which factors predict optimal pubertal development, the most effective markers to measure tempo and efficacy of treatment and ultimately how this may affect fertility and pregnancy outcome. I first present the studies assessing the tools to monitor uterine and breast development and then those examining treatment outcomes.

ABSTRACT SUMMARY

Study 1

A prospective study examining the reproducibility of transabdominal ultrasound and agreement with transvaginal ultrasound and Magnetic Resonance Imaging.

Study 2

A prospective study exploring the development of a novel technique using 3D imaging to determine change in breast volume.

Study 3

A retrospective cross-sectional study examining uterine size in those who had pubertal induction treatment compared to those who underwent spontaneous puberty.

Study 4

A prospective observational study examining the variability of uterine and breast parameters in those undergoing pubertal induction.

Study 5

A prospective observational study examining the application of 3D breast imaging in trans females undergoing oestrogen treatment.

Study 6

A retrospective cross-sectional study examining the fertility and pregnancy outcomes in women with Turner syndrome and Premature ovarian insufficiency.

CONCLUSION

These studies have shown that variation in uterine and breast development with oestrogen treatment can be tracked continuously with ultrasound and 3D breast imaging, respectively. Better individualisation of treatment may result in improved outcomes in final volume of oestrogen target organs which may impact future fertility.

FULL THESIS ABSTRACT

STUDY 1 - EXAMINATION OF THE REPRODUCIBILITY OF UTERINE DIMENSIONS USING TRANSABDOMINAL ULTRASONOGRAPHY

Study objective

Accurate assessment of uterine size is essential to provide clinical information in several gynaecological and endocrinological presentations with repeated review often indicated in pubertal induction treatment. Transabdominal ultrasound (TAUS) is usually first line in younger females but is subject to variation due to patient and practitioner factors which may limit its use for comparative or serial measurements. Little data is available regarding the reproducibility of TAUS. I therefore wished to explore the reproducibility of TAUS using a standardised protocol and the agreement with transvaginal ultrasound (TVUS) and Magnetic Resonance Imaging (MRI).

Study design

A single centre prospective observational study

Methods

Ultrasound examinations took place at University College London Hospital (UCLH) and were completed by myself (EB) and a colleague, following a standardised protocol. The TAUS examinations were either completed by both observers to assess interobserver variability (18 participants) or by myself twice to assess intraobserver variability (55 participants). To examine agreement between TAUS and TVUS / MRI, patients had uterine measurements completed

with both imaging modalities (TAUS vs TVUS 10 subjects and TAUS vs MRI 15 participants).

Results

There was no significant difference for uterine length, uterine body anterior posterior, transverse and uterine volume measurements when assessing interobserver and intraobserver reproducibility of TAUS and the intraclass correlations (ICC) were >0.9 for all parameters. There was generally poor ICC with greater variability for the comparison of TAUS and TVUS and poor ICC between TAUS and MRI.

Conclusion

Excellent reproducibility for TAUS, when completed by experienced practitioners and following a standardised protocol, can be achieved providing reassurance for its first line use in those requiring assessment of uterine size. Due to poor ICC between modalities interchanging between TAUS and TVUS or MRI for uterine size should not be advocated.

STUDY 2 - CHALLENGES IN DEVELOPING A QUANTITATIVE METHOD OF MEASURING BREAST DEVELOPMENT USING 3D IMAGING - AN EXAMPLE OF A NOVEL METHOD FOR USE IN INDUCED BREAST DEVELOPMENT WITH EXOGENOUS OESTROGEN

Study objective

Optimal breast development is an essential part of exogenous oestrogen treatment in females undergoing pubertal induction. To date breast assessment in pubertal induction is completed by subjective Tanner staging visual

assessment and there is no current objective quantitative breast volume assessment utilised in this cohort of females. I set out to develop a novel technique using 3D imaging to determine change in breast volume that is applicable when no pre-existing breast contours are present, which would permit longitudinal quantitative assessment of breast differential volume during pubertal induction. I also examined the feasibility and reproducibility of anatomical landmarking.

Study design

A single centre prospective observational study.

Methods

The imaging methodology was developed using a single male subject to assess reproducibility and validity. Breast images were taken using a 3D photographic system. Reproducibility of landmarking was trialled with different techniques using breast images from 14 individuals who had completed pubertal induction. For breast volume assessment two images, taken at different times, were manually superimposed to produce a differential breast volume. The initial step of method development set out to show that volume change was not secondary to positioning artefact or image manipulation. The technique was then used to assess reproducibility in 29 participants undergoing induced breast development with exogenous oestradiol.

Results

Good intraobserver reproducibility (intraclass correlation (ICC) 0.77) was demonstrated with static image manipulation. Validity of the imaging technique was established as there was no significant difference between the known

reference volume produced by computer generated warping and that calculated by manual image manipulation. There was excellent intraobserver reproducibility for breast volume calculation in participants undergoing induced breast development (ICC 0.99). There was poor reproducibility for landmarking for all different techniques trialled.

Conclusion

3D imaging is a promising novel tool to provide quantitative breast volume assessment in individuals undergoing breast induction with exogenous oestradiol treatment.

STUDY 3 - UTERINE VOLUME AFTER INDUCTION OF PUBERTY IN WOMEN WITH HYPOGONADISM

Study objective

Adequate uterine growth is an essential component of pubertal induction. I wished to assess uterine size in women who have undergone induction of puberty having presented with primary amenorrhoea from a variety of causes. I set out to refine the knowledge base in this area by recording ultrasound data from a single observer, documenting a reference group for comparison, and exploring clinical parameters which may determine adult uterine size.

Study design

A single centre retrospective cross-sectional study

Methods

Ninety-five women with hypogonadism who had previously undergone pubertal induction and were receiving maintenance oestrogen replacement as adults were recruited. The reference group consisted of 35 nulliparous women attending with male factor subfertility with a normal pelvis on ultrasonography. Pelvic ultrasound was performed by a single observer. Uterine dimensions (total length, anterior posterior (AP), transverse, uterine volume and fundal cervical AP ratio (FCR) measurements) were recorded. Clinical details were also recorded.

Results

Those with hypogonadism and induced puberty had significantly reduced uterine dimensions compared to the reference group (uterine length 64 mm vs 71mm p = < 0.05, uterine volume 28.9ml vs 43.9ml p = < 0.05). All women in the reference group attained a mature uterine configuration with an FCR >1, compared to 84% of those with hypogonadism (p = 0.01). 24% and 48 % of the diagnostic group had total uterine length and uterine volume measurements less than the 5th percentile of the reference group respectively. In a subgroup of 22 women in whom serum oestradiol concentrations could be analysed, there was a positive correlation between this parameter and uterine volume.

Conclusion

Despite standard oestrogen therapy, uterine growth is often compromised in those with hypogonadism. Uterine health has historically been overlooked in pubertal induction protocols however with increasing options for fertility treatment, adequate uterine development is crucial. Given the variation in uterine

size witnessed, a more tailored approach to treatment with regular monitoring of uterine dimensions should be advocated.

STUDY 4 - VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM

Study objective

Induction of puberty with exogenous oestrogen results in considerable variability in final uterine and breast volumes and therefore I set out to quantify the variability of these two outcome measures with a view to establishing monitoring methods that could be used to individualise treatment protocols.

Study Design

A single centre prospective observational study.

Method

Sixteen participants with pubertal delay and primary amenorrhoea, due to hypogonadism underwent pubertal induction using a standardised treatment protocol of transdermal 17β oestradiol (17βE) (Evorel™). The starting dose was 12.5mcg which increased to 25mcg (patch changed twice weekly) after 4 months. After 8 months of treatment there was a divergence in oestradiol dose. Follow up was every 2 months for a total of 8 months and at each follow up uterine dimensions using ultrasound, hormonal concentrations, anthropometric measurement, bone indices and breast development assessed by both Tanner staging and 3D photographic imaging were assessed.

Results

After 8 months of treatment, the changes in oestradiol concentrations (0 - 174 pmol), uterine volume growth (4.4 – 16.4mls) and breast volume (1.76 - 140.1mls) varied greatly between individuals. Of uterine parameters, transverse uterine diameter was most closely associated with serum oestradiol levels at 8 months (beta standardised coefficient = 0.80 p = 0.001). Change in breast volume was associated with age of treatment initiation (beta standardised coefficient 0.55 p = 0.04).

Conclusion

I demonstrate variation in response to exogenous oestrogen, emphasising the necessity for individualised dose titration. In the absence of sensitive oestradiol assays, uterine transverse measurements may be used as a surrogate marker of oestrogen sensitivity to guide early dose adjustment. 3D breast imaging may provide a quantitative assessment of breast development to complement Tanner breast staging.

STUDY 5 - QUANTITATIVE ASSESSMENT OF BREAST VOLUME USING 3D IMAGING IN TRANS FEMALES: A PILOT STUDY

Study objective

There is little research exploring the natural history of breast development in trans females undergoing exogenous oestrogen treatment, especially in the adolescent age group. Therefore, I wished to explore the application of 3D breast imaging in this cohort to provide a quantitative assessment of breast development and volume change with treatment.

Study design

A single centre prospective observational study.

Methods

Four participants undergoing induced breast development with exogenous oestradiol were recruited. 3D breast images were taken prior to oestradiol treatment and after 6 months of use and differential breast volume was calculated.

Results

Following 6 months of treatment the differential breast volume ranged between 5.2mls and 15.3mls

Conclusion

I demonstrate the successful application of 3D breast imaging and a novel measurement technique in trans females, not requiring anatomical landmarking, to provide differential breast volume measurements.

STUDY 6 - FERTILITY AND PREGNANCY OUTCOMES IN WOMEN WITH TURNER SYNDROME AND PREMATURE OVARIAN INSUFFICIENCY

Study objective

I wished to examine the fertility and pregnancy outcomes in women with Turner syndrome (TS) and premature ovarian insufficiency (POI) undergoing oocyte donation (OD) treatment or spontaneous pregnancy. In particular, I sought to compare the maternal outcomes in our cohort who received regular health

surveillance and cardiology review, with previously reported literature. In addition, I was able to compare OD pregnancy outcomes in TS with those in women with idiopathic POI attending UCLH.

Study design

A single centre retrospective cross-sectional study.

Methods

Seventy-four women with Turner syndrome (TS) underwent oocyte donation (OD) treatment with a total of 105 pregnancies and 31 women with TS had 71 spontaneous conceptions. Fifty women with premature ovarian insufficiency (POI) underwent oocyte donation and there were 84 pregnancies and 18 women had spontaneous pregnancies. Fertility outcomes included clinical pregnancy and live birth rate. Pregnancy outcomes included miscarriage rate, prevalence of hypertension (HTN), gestational diabetes (GDM), lower segment caesarean section (LSCS), small for gestational age (SGA), prematurity and vertical transmission of Turner syndrome.

Results

In those with TS, OD pregnancies were associated with increased rates of LSCS and SGA compared to spontaneous pregnancies; LSCS (OR 4.19 95% CI 1.61 – 10.8 p = 0.003) and SGA (OR 2.92 95% CI 1.02 - 8.38 p = 0.04). There were no recorded cardiac events but 5 (17.2%) cases of vertical transmissions of TS in daughters were identified. Oocyte donation in those with TS was associated with lower live birth rate per cycle started (OR 0.53 95% CI 0.34 - 0.84 p = 0.008) and a higher rate of miscarriage compared to women with POI (40% vs 26.2% p = 0.04). Compared to population data in women with TS and spontaneous

conception the rate miscarriage was higher as was the rate of HTN pathology (12.5% vs 2.9% p = <0.001), GDM (15% vs 3.7% p = <0.001), LSCS (51.2 % vs 16.9% p = <0.001) and SGA (15.8 % vs 2.7% P = <0.001).

In those with POI and spontaneous conceptions the miscarriage rate was not higher than reference data.

Conclusion

I show that pregnancy in women with TS, whether OD or spontaneously conceived, carries obstetric risks and therefore women with TS, considering pregnancy, should receive comprehensive pre pregnancy counselling and optimal obstetric care.

IMPACT STATEMENT

Many females who do not undergo spontaneous puberty will require oestrogen treatment and pubertal induction. The transition through puberty is important for both psychological and physical wellbeing and inadequate treatment can leave dissatisfaction in breast size and shape and an immature uterine size may have long term sequelae. There is no clear consensus as to a unified pubertal induction protocol and clinical practice varies. Importantly given the variation in response seen, an individualized dosing schedule should be advocated moving away from a didactic schedule for all.

University College London Hospital (UCLH) is a centre of excellence treating many females with different pathologies requiring pubertal induction treatment and long-term adult care. My research has provided greater understanding about the factors affecting variation in response to treatment, and the best markers to use clinically to guide treatment dosing. This will provide insight and confidence in this field. With breast development being so important for psychological wellbeing for many, I recognised the deficiency in quantitative clinical assessment tools and set out to develop a novel technique with 3D breast imaging to understand the natural history of breast development with exogenous oestrogen in greater depth. The latter will hopefully be developed further and, in the future, may become a routine clinical tool adding significant benefit.

Whilst my research has focused on females with genetic and idiopathic forms of hypogonadism the findings are applicable to a wider group of patients. There is an ever-increasing number of survivors of childhood cancer, a proportion of whom will have ovarian insufficiency. Optimising pubertal induction outcomes in

this group may improve long term psychological sequelae after cancer survivorship.

With increasing numbers of women who have been through pubertal induction seeking fertility and reproductive options, comprehensive, evidence based, up to date clinical counselling regarding the potential maternal and fetal implications is paramount. My research has provided further clarification regarding fertility and pregnancy outcomes in those with Turner syndrome and premature ovarian insufficiency. This will allow patients to make informed choice and guides suitable pre pregnancy and antenatal care to ensure optimal safety for these women.

My work to date, that has been shared within the academic world has been positively received and has stirred much interest at national and international conferences. The list of my publications and presentations can be seen below. Based on my research, I have subsequently been invited to peer review journal articles within a similar field. Furthermore, my work has provided the motivation for many new projects within UCLH.

As a Gynaecologist in the world of Endocrinology my thesis work has now set the scene to allow a multidisciplinary dedicated pubertal induction clinic which will allow holistic care for patients and provide a wealth of research potential. This research necessitated a dedicated induction of puberty clinic to allow for systematic collection of data. This clinic has proven to be so important for patient satisfaction and for refinement of our treatment program that the Trust has now adopted the model for a specialist service.

PUBLICATIONS, PRESENTATIONS AND AWARDS

The Copyright agreement documents granting permission for the below publications to be used in my Doctoral thesis can be seen in Appendix 1. The UCL research paper declaration forms can be seen below. The final publications can be found in Appendix 2.

PUBLICATIONS

- Burt E, Davies MC, Yasmin E, Cameron-Pimblett A, Talaulikar V, La Rosa C, Clarke SA, Conway GS. Fertility and pregnancy outcomes in women with Turner syndrome: A single centre experience. Clin Endocrinol (Oxf). 2024 Jul;101(1):51-59

This publication is based on study 6.

- Burt, E., & Yasmin, E. (2023). Primary Amenorrhoea and Delayed Puberty (pp. 57-66). In Naomi S. Crouch, Cara E. Williams (Eds.), *Paediatric and Adolescent Gynaecology for the MRCOG* © Naomi S. Crouch and Cara E. Williams 2023, published by Cambridge University Press.

This publication is found in the thesis introduction.

- Burt E, Ruff C, Yasmin E, Davies M, Cameron-Pimblett A, Butler G, Conway G. Challenges in developing a quantitative method of measuring breast development using 3D imaging: An example of a novel method for use in induced breast development with exogenous oestrogen. Clin Endocrinol (Oxf). 2023 Jan;98(1):68-73.

This publication is based on study 2.

- Burt E, Yasmin E, Davies MC, Creighton S, Brain C, Ruff C, Learner HI, Williams L, Cameron-Pimblett A, Talaulikar V, Conway G. Variability of response to early puberty induction demonstrated by transverse uterine diameter measurement and a novel method of 3D breast imaging. Clin Endocrinol (Oxf). 2022 Jul;97(1):91-99.

This publication is based on study 4.

- Burt E, Davies MC, Yasmin E, Cameron-Pimblett A, Mavrelos D, Talaulikar V, Conway GS. Reduced uterine volume after induction of puberty in women with hypogonadism. Clin Endocrinol (Oxf). 2019 Dec;91(6):798-804.

This publication is based on study 3.

ORAL PRESENTATIONS

- Pregnancy outcome in Turner syndrome: single centre experience. British Fertility Society Annual general meeting. 2021. Oral presentation

- Adult uterine size depends on pubertal oestrogen. Annual Update in Paediatric and Adolescent Gynaecology. Joint RCOG/BritSPAG Meeting. 2018. Oral presentation.

- Adult uterine size depends on pubertal oestrogen. RCOG World Congress. 2018. Oral presentation.

- Adult uterine size depends on pubertal oestrogen. British Fertility Society Annual general meeting. 2018. Oral presentation.

- Uterine Development: the effect of induction of puberty with oestrogen in primary amenorrhoea. British Society for Paediatric Endocrinology & Diabetes. 2017. Oral presentation.

- Uterine Development: the effect of oestrogen induction of puberty in primary amenorrhoea. 14th European congress of paediatric and adolescent gynaecology. 2017. International oral presentation.

POSTER PRESENTATIONS

- Pregnancy Outcomes in Turner Syndrome – Single Centre Audit. ESHRE.2020. Poster presentation.

- Reproductive Life Course Project: Preliminary data from UK Turner Syndrome Pregnancy audit. British Endocrine Society. 2018. Poster presentation
- Uterine Development: The Effect of Oestrogen Induction of Puberty in Premature Ovarian Insufficiency. British Menopause Society 2017. Poster presentation

AWARDS

- Adult uterine size depends on pubertal oestrogen. Annual Update in Paediatric and Adolescent Gynaecology. Joint RCOG/BritSPAG Meeting. 2018. Best oral presentation.

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1. **For a research manuscript that has already been published** (if not yet published, please skip to section 2)

   a) **What is the title of the manuscript?**

   Fertility and pregnancy outcomes in women with Turner syndrome: A single centre experience

   Please include a link to or doi for the work

   doi: 10.1111/cen.15078

   b) **Where was the work published?**

   Clinical Endocrinology

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   Wiley

   d) **When was the work published?**

   May 2024

   e) **List the manuscript's authors in the order they appear on the publication**

   E Burt, M C Davies, E Yasmin, A Cameron-Pimblett, V Talaulikar, C La Rosa, S A Clarke and G S Conway

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3. **For multi-authored work, please give a statement of contribution covering all authors** (if single-author, please skip to section 4)

   E Burt – Study conception and design, acquisition of data, analysis and interpretation of data. Drafting the manuscript and final approval of the version to be published

   Professor Conway – Study concept and design and revising the article critically for important intellectual content

   All other authors - Revising the article critically for important intellectual content

4. **In which chapter(s) of your thesis can this material be found?**

   Study 6 – Abstract, Introduction, Methodology, Results and Discussion

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i) **What is the title of the manuscript?**

Primary Amenorrhoea and Delayed Puberty

Please include a link to or doi for the work

https://doi.org/10.1017/9781108907507.009

j) **Where was the work published?**

Paediatric and Adolescent Gynaecology for the MRCOG

k) **Who published the work?** (e.g. OUP)

Cambridge University Press

l) **When was the work published?**

2023

m) **List the manuscript's authors in the order they appear on the publication**

Burt E and Yasmin E

n) **Was the work peer reviewed?**

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8. **For multi-authored work, please give a statement of contribution covering all authors** (if single-author, please skip to section 4)

   E Burt –Drafting the manuscript and final approval of the version to be published.

   E Yasmin - Revising the manuscript critically for important intellectual content**.**

9. **In which chapter(s) of your thesis can this material be found?**

   Chapter 1 - Introduction

10. **e-Signatures confirming that the information above is accurate** (this form should be co-signed by the supervisor/ senior author unless this is not appropriate, e.g. if the paper was a single-author work)

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    Supervisor/ Senior Author (where appropriate)

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a) **What is the title of the manuscript?**

Challenges in developing a quantitative method of measuring breast development using 3D imaging: An example of a novel method for use in induced breast development with exogenous oestrogen

Please include a link to or doi for the work

doi: 0.1111/cen.14815

b) **Where was the work published?**

Clinical Endocrinology

c) **Who published the work?** (e.g. OUP)

Wiley

d) **When was the work published?**

January 2023

e) **List the manuscript's authors in the order they appear on the publication**

Burt E, Ruff C, Yasmin E, Davies M C, Cameron-Pimblett A, Butler G and G S Conway

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    Clifford Ruff - Study concept and design and revising the article critically for important intellectual content.

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Burt E, Davies M C, Yasmin E, Cameron-Pimblett A, Mavrelos D, Talaulikar V, Conway G S

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    Dimitri Mavrelos - Acquisition of data

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STATEMENT OF PERSONAL CONTRIBUTION

My personal contribution to the studies contained in this thesis is presented below. I was fortunate to have the support and guidance of my supervisors and other colleagues who were able to assist me in the more specialist aspects.

Research protocol design

For each study I was responsible for designing and completing the research proposal and protocol. Research meetings were held on a regular basis to further discuss the research protocols in collaboration with the supervisory team and the wider clinical team, and where necessary, adjustments made. I liaised closely with members of the extended multidisciplinary team to ensure investigations could occur in line with the research protocol for example radiology department and consultant Radiologist for MRI examinations and nuclear medicine team for bone density scans. With regards to the breast imaging, the clinical protocol was created with the input of the principal medical physicist.

Ethics

I completed the ethical application with collaboration of the Research and Development department at UCLH for studies 2, 4 and 5. All supporting documentation including patient information sheets, participants invitation letters and consent forms was designed and written by myself and can be seen in Appendix 3. As the study included both participants under and over the age of 16 additional documents were required for parents/guardians of those under the age of 16 at recruitment. I ensured rigorous completion and archiving of consent documents.

For studies 1, 3 and 6 there was pre-existing department wide ethical approval for Turner Life Course Project (TLCP) and Reproductive Life Course Project (RLCP) for questionnaire and clinical data analysis. I sought consent for any individuals who had not previously taken part in earlier studies.

I was responsible for reporting all ethics updates.

Pelvic ultrasound protocol

I developed a protocol for the transabdominal and transvaginal scanning. This was with collaboration of the other experts in paediatric scanning. The protocol included details of participant preparation, scan machine setting, acquisition of measurements including the agreed anatomical landmarks to be used and necessary documentation to ensure that these investigations were standardised. The details of the protocol are described in detail in Study 1 methodology. I completed all pelvic ultrasound examinations, both transabdominal and transvaginal, required for studies 1, 3 and 4.

I reviewed all the ultrasound images for Study 6 which had been performed as part of routine care. I examined the ultrasound images for all those who had had fertility treatment and pregnancy. For those who had a scan I applied the standardised measurement and marked these on the images myself.

3D breast scanning

I developed a novel technique for the completion of 3D breast scanning and volume measurement. This was in collaboration with principal medical physicist, Cliff Ruff. The 3dMD breast scanner was originally designed for facial work and therefore I had to plan the necessary changes for adaptation for the breast, including participant positioning and image acquisition and optimising image

quality in collaboration with Cliff Ruff. I examined multiple acquisition techniques and designed a postural support which was manufactured by the workshop team. The final protocol included details of participant preparation and positioning, machine setting and calibration, acquisition of images and manipulation of images. The detail of the protocol is described below in Study 2 methodology. All breast images were taken by myself and I undertook the calibration 3dMD Photogrammetric System. I performed all image manipulation, marking for reproducibility and volume assessment. Cliff Ruff aided me with any technical issues, and he was also responsible for designing the software and computer programming necessary for the work. Cliff Ruff was pivotal in proposing all mathematical models and adjusting existing software packages.

Participant recruitment

I was responsible for and completed all patient recruitment. I reviewed clinic lists and medical notes to ensure suitable and optimal patient number recruitment. Recruitment was challenging as I was required to attend multiple clinics as there is no dedicated common referral pathway into paediatric endocrinology/ gynaecology and reproductive endocrinology clinics. I provided the necessary patient information sheets, discussed the research detail with the patients and ensured the consent forms were signed.

Clinical follow up

I completed the clinical reviews at the initial presentation and when participants were returning for follow up, again necessitating my attendance at multiple different clinics in the Paediatrics and Gynaecology departments. I took clinical histories and performed the necessary physical examinations, including that of body fat composition.

I was responsible for the completion of all structured telephone interviews and completed detailed note review where necessary.

Phlebotomy

For the tests completed within the hospital laboratory, the blood tests were taken by the routine hospital department using the standard protocol. For the sensitive oestradiol assay which was to be processed at South Manchester Hospital biochemistry laboratory using a sensitive LC-MS/MS (Liquid chromatography tandem mass spectrometry) method, I was responsible for the blood taking, processing, sample labelling and storage in a dedicated research laboratory. The samples were then appropriately labelled and stored in vials in compliant laboratory freezers (as per laboratory instruction). I was responsible for organising the suitable courier (as per laboratory instruction) to transport the samples to South Manchester Hospital (Wythenshawe).

Data analysis

I was responsible for all data collection and designed the database and was responsible for all data entry. I completed all the statistical analysis myself and interpreted all the results. I sought clarification from my supervisor where necessary.

Presentations and publications

I completed all the abstract submissions and poster and oral presentations of the work presented to date. I delivered all the oral and poster presentations myself at both national and international conferences. For the papers generated from studies 2,3,4 and 6 I was responsible for the completion of all the manuscript

drafts of the work published to date. I liaised and communicated with the journal editorial team.

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AFC	Antral follicle count
ALP	Alkaline phosphatase
AMH	Anti-Mullerian Hormone
ASI	Aortic size index
BMC	Bone mineral concentration
BMD	Bone mineral density
BMI	Body Mass Index
BSA	Body surface area
CDGP	Constitutional delay of growth and puberty
CEE	Conjugated equine oestrogen
CHH	Congenital hypogonadotrophic hypogonadism
COCP	Combined oral contraceptive pill
DEXA	Dual emission x-ray absorptiometry
DSD	Differences of sex development
E2	Oestradiol
EE	Ethinylestradiol
ER	Oestrogen receptor
ESHRE	European Society of Human Reproduction and Embryology
FCR	Fundus AP: Cervix AP ratio
FISH	fluorescence in situ hybridization
FSH	Follicle Stimulating Hormone
FTET	Frozen thawed embryo transfer
GD	Gonadotrophin Deficiency
GDM	Gestational diabetes
GH	Growth Hormone
GnRH	Gonadotrophin Releasing Hormone

GPER1	G protein-coupled estrogen receptor 1
HA	Hypothalamic amenorrhoea
HELLP	Haemolysis, elevated liver enzymes and low platelets syndrome
HH	Hypogonadotrophic hypogonadism
HIV	Human immunodeficiency virus
HP	Hypopituitarism
HPO	Hypothalamic Pituitary Ovarian
HRA	Health Research Authority
HRT	Hormone Replacement Therapy
HWR	Hip waist ratio
ICC	Intraclass correlation
ICSI	Intracytoplasmic sperm injection
IGF-1	Insulin Like Growth Factor 1
IMF	Infra Mammary Fold
IRAS	Integrated Research Application System
IUD	Intrauterine death
IUGR	Intrauterine growth retardation
IVM	Invitro oocyte maturation
LH	Luteinising Hormone
LSCS	Lower segment caesarean section
MRI	Magnetic resonance imaging
MRKH	Mayer-Rokitansky-Küster-Hauser syndrome
OS	Ovarian stimulation
OTC	Ovarian tissue cryopreservation
PCOS	Polycystic ovarian syndrome
PET	Preeclampsia
PGT-A	Preimplantation genetic testing for aneuploidy

PI	Pulsatility index
PIH	Pregnancy induced hypertension
POI	Premature Ovarian Insufficiency
RCT	Randomised controlled trial
RLCP	Reproductive Life Course Project
SGA	Small for gestational age
TAUS	Transabdominal ultrasound
TD	Transdermal
TLCP	Turner Life Course Project
TOP	Termination of pregnancy
TVUS	Transvaginal ultrasound
UCLH	University College London Hospital
US	Ultrasound

CHAPTER 1

LITERATURE REVIEW

FEMALE PUBERTY

Female puberty, mediated predominantly by oestradiol, is a slow linear process requiring approximately 3- 5 years for completion. Full transition from childhood to adulthood with reproductive potential is dependent on an appropriately functioning hypothalamic - pituitary - ovarian (HPO) axis.

Puberty culminates in the development of secondary sexual characteristics. Breast maturation, changes in bodily shape with growth and presence of axillary and pubic hair are the external phenotypic changes associated with puberty, whilst the attainment of an adult uterine configuration and many more subtle changes, including those affecting the cardiovascular and neurological systems are internal alterations (1). Puberty sees significant physical, psychological and social alterations.

In general, thelarche with breast budding is usually the earliest sign of puberty, followed by growth velocity, with menarche occurring 2-3 years after the onset of puberty. Whilst the onset, tempo and sequence of puberty will vary between individuals, delay in initiation of events or arrest during the process warrants further investigations. Pubertal transition requires not only an intact HPO axis but also functional reproductive organs.

FEMALE PUBERTY AND HYPOTHALAMIC PITUITARY OVARIAN AXIS

The development of the Hypothalamic - Pituitary - Ovarian (HPO) axis occurs in utero and it becomes fully active by the second trimester. In the latter stages of

gestation, the hormonal axis remains suppressed due to the negative feedback of maternal and placental hormones (2). Prior to puberty, females are not oestrogen naïve, as with escape of this inhibition after birth, there is reactivation of the neonatal HPO axis with a hormonal surge of Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH) and oestradiol (E2). A peak level of oestradiol occurs at 12-18 months of age and is responsible for the relatively larger uterine size and evidence of follicular ovarian activity witnessed during this early postnatal period (3). Furthermore, secondary to this hormonal exposure, there may be some evidence of breast tissue development (1, 4). This is often referred to as a 'mini puberty' (2). In females with an aberration in their HPO axis, the lack of this hormonal surge may herald the first signs predicting hormonal status in the later years (4, 5). In contrast in those with hypogonadism there may be an exaggerated FSH response (6). It is being explored if clinical and biochemical evidence during these early months may provide useful insight for later years (7).

The HPO axis remains dormant during infancy and hormonal levels again decline until the onset of puberty. The HPO axis is reawakened at approximately 6-8 years of age. The exact mechanisms of how the HPO axis is activated remains to be fully elucidated. Primarily there is diurnal variation with nocturnal activity and oestradiol production, however as puberty proceeds, secretion both day and night occur (8, 9). Pulsatile gonadotrophin releasing hormone (GnRH), secreted from the hypothalamic arcuate nucleus, in turn stimulates the pituitary gland to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). Initially FSH levels are higher than LH levels. The FSH and LH regulate the ovarian production of sex steroids according to the two-cell–two-gonadotropin theory and paracrine signalling in the ovarian follicle (10, 11). FSH triggers ovarian folliculogenesis with proliferation of the granulosa cells, and in parallel, LH

stimulates theca cell development and steroidogenesis in the ovarian theca cell compartment. The resultant androstenedione diffuses from the theca to the granulosa cells where aromatization occurs, under the influence of FSH, culminating in 17β-oestradiol production. Anti-Mullerian Hormone (AMH) produced by the follicular granulosa cells of the ovary remains relatively stable during puberty (4). There is maturation of the HPO axis and subsequent coordinated hormonal patterns associated with ovulation and menstruation.

OESTROGEN RECEPTORS

Oestradiol, a lipid-soluble steroid hormone, exerts its biological actions via three oestrogen receptor (ER) subtypes affecting different biological signalling cascades. The ERα and ERβ are both intracellular nuclear bound steroid receptors which activate and regulate genomic transcription. A third, G protein-coupled, estrogenic receptor 1 (GPER1) is located on the cell surface and initiates protein - kinase pathways (12). The genes ERα and ERβ are located on the long arm of chromosome 6 (6q25.1) and chromosome 14 (14q23.2) respectively and GPER1 is encoded on chromosome 7 (7p22.3) (13).

ERα receptors are predominately found in the uterus, breast, ovary, epididymis, liver, kidney, adipose tissue, bone and brain. ERβ receptors are located in the ovary, bone, endothelium, lung, bladder, brain and gastrointestinal tract. GPER1 is widely distributed in various tissues.

ERα and ERβ receptor functions are affected by their respective isoforms as well as their relative distribution in the target tissue. Epigenetic mechanisms may, in turn, further affect the gene expression of ERs (12).

OVARIAN DEVELOPMENT

In the female, oogenesis and the development of primordial follicles is completed by 18-22 weeks' gestation with oocytes arrested in meiosis at prophase 1. At birth there are appropriately 300,000 follicles per ovary and this declines until the age of menopause (14). During puberty there is ovarian growth and the detection of apparent follicles seen on ultrasound (15). Many authors who describe the normal uterine growth trajectory have also used their study population to comment on the ovarian changes with an increase in volume during puberty (3, 16-19). The ovarian volume in prepubertal girls is approximately 1-2 mls and increases to 6-10mls at Tanner stage 5 (15, 18, 20, 21). Ovarian volume will vary in size with the menstrual cycle (15, 16).

BREAST DEVELOPMENT AND THELARCHE

Rudimentary breast development occurs in utero as early as the 5th week of gestation when a 'milk streak' is identifiable. The milk streak is an ectodermal thickening which runs from the axilla to the groin, and whilst it persists in the thoracic region to form the 'milk ridge' the remainder of the milk streak regresses (22). The milk ridge forms the lactiferous ducts and glandular tissue (23). The nipple develops at 36 weeks' gestation. At birth there may be varying levels of morphological differentiation, hypertrophy and secretory activity under maternal hormonal stimulation but thereafter the glands involute and remain quiescent in infancy (22, 23).

The most noticeable breast transformation occurs during puberty and is oestrogen dependent. With thelarche and maturation of the breast tissues there is differentiation and elongation of the epithelial ducts and development of the terminal duct-lobular units. Maturation of the breast sees a connective tissue

framework enclosing adipose and glandular components. Mammogenesis is dependent on the delicate balance between oestrogen and progesterone producing ductal proliferation and vertical lobular growth with alveolar budding respectively (24, 25). Similarly, to the other reproductive organs and bone, Growth Hormone (GH) and Insulin Like Growth Factor 1 (IGF-1) are also necessary. Growth Hormone (GH) and Insulin Like Growth Factor 1 (IGF-1) are both adjuvants to proliferation, acting in a synergistic fashion with oestradiol (26). Adipose tissue is a significant part of adult breast tissue and contributes to final breast volume (27).

Breast budding is usually the first sign of puberty (27). Thelarche and the development of a mature and functional mammary gland composed of adipose, lobules and duct cells with the external appearances of the nipple and areola takes approximately 4 years for full completion. Initial breast development is often asymmetrical. The average age of thelarche (breast development Tanner Stage $\geq$ B2) is 11.3 years and can range between 8 to 13 years of age. The exact timing is affected also by ethnicity and Body Mass Index (BMI) (28, 29) and appears to be starting earlier (30). Full breast development continues until 18-20 years of age (27).

ADRENARCHE, GROWTH AND BODY COMPOSITION

Adrenarche, with the arrival of pubic and axillary hair occurs due to adrenal production of androgens. This occurs independently to the oestradiol driven aspects of puberty.

The hormonal milieu during the pubertal years is responsible for the associated growth spurt, epiphyseal closure and acquisition of peak bone mass. Cortical bone predominantly contains ERα receptor whereas trabecular bone contains

both oestrogen receptor (ER) types (ERα and ERβ). Peak bone velocity usually occurs between Tanner breast stages 3 and 4 (28).

Oestradiol is not solely responsible for bone growth and maturation as Growth Hormone (GH) and Insulin-like Growth Factor 1 (IGF-1) are both critical (8). Oestradiol has a direct effect on bone but also stimulates the release of GH and subsequently IGF-1, which propagates and augments the effects of oestradiol. Both oestrogen receptor subtypes are expressed on both the anterior pituitary and the hypothalamus (31). An increase in GH can be seen in early puberty and peaks at Tanner stage 3-4 (32) and prior to the onset of the pubertal years the GH/ IGF-1 axis cause the steady bone growth seen (33).

Oestrogens have a biphasic effect on the long bones (34). At low oestrogen concentration growth is induced but at higher oestradiol concentrations there is epiphyseal closure. In the early pubertal years, under modest morning oestradiol exposure (oestradiol concentrations between 13 and 31pmol/l) (35), there is acceleration of bone growth and mineralization, with approximately 20-25cm additional height being attained during these years (1, 8). With increasing oestradiol exposure as puberty continues, a plateau in growth rate is witnessed with maturation of the long bone growth plates. Growth in females occurs until the age of approximately 16 (36). Variation in growth between individuals may be due to several factors including ERα receptor polymorphisms (37).

Oestrogen is not only required for bone linear growth but also plays a fundamental part in the regulation in bone turnover affecting osteoclast differentiation and function and ultimately bone mineral density (BMD). During the pubertal years there are high levels of bone turnover markers, including bone Alkaline phosphatase (ALP) reflecting the increased bone metabolism and an

increased growth velocity during puberty. There is an increase in both trabecular bone within the epiphyseal growth plates and an increase in cortical bone. Oestrogen stimulates osteoblast formation and has a proapoptotic effect on osteoclasts pushing the balance of remodelling towards increased bone formation. Bone accrual occurs mid to late puberty and there is a doubling of the bone mass with females gaining approximately a third of their peak BMD within the pubertal years (33). Thereafter the markers of bone metabolism begin to decline as there is closure of the epiphyseal plates (36). Peak rates of bone mineralisation lag behind peak growth velocity by about 8 months (38).

Concern has been raised when oestradiol treatment promotes supraphysiological concentrations as this could lead to detrimental effects on the bone development and final height due to premature epiphyseal closure (39, 40).

Anthropometric measurements, under the influence of sex steroids during puberty, are altered, with changes in the female body shape, composition and adipose distribution. The majority of the literature supports the idea of an increase in body fat mass during the pubertal years (41-43). Goulding et al. found that between Tanner stages 1-5, there was an increase in truncal fat mass and a decrease leg fat mass when examined with dual energy X ray- absorptiometry supporting the notion in change in body shape (41). Reinehr also demonstrated an increase in BMI over the pubertal years (44).

PUBERTY AND THE UTERUS

One of the fundamental effects of oestrogen during puberty is to promote development of the uterus in preparation for future fertility. The uterus grows from a small tubular structure to obtain a heart-shaped mature configuration increasing in size in all dimensions.

During the postnatal period, with loss of the influence of the maternal and placental endocrine milieu on the fetal HPO axis, there is oestradiol production and early priming of the uterus. The implications of this very early uterine growth and lack of it in those with hypogonadism is not fully realised. Sonographically, the uterus and ovaries appear comparably large and the endometrium can be visualized (3, 45) however, thereafter uterine and ovarian growth remains latent.

There is evidence of uterine growth prior to menarche and thelarche starting at the age of approximately 6-8 years of age (15, 21). It has been postulated that prepubertal uterine growth may have an ultimate influence on final uterine growth potential. Prior to the main surge in oestrogen during puberty, the growth predating the principal pubertal years, is thought to be secondary to adrenal steroidogenesis, ovarian follicles producing low oestradiol levels, temporary fluctuation of ovarian oestradiol or other adjuvant hormones such as growth hormone and IGF-1 (46). Independence of sustained oestradiol stimulation is evidenced by the fact that uterine growth occurs without concomitant breast development and interestingly the uterine size in prepubertal females was found to be correlated with height (20, 47). The somatotrophic axis is vital for pubertal development with GH receptors located in the endometrium and myometrium (48) as evidenced in women with growth hormone deficiency having delayed pubertal development (49). Furthermore, in those with isolated growth hormone deficiency or GH receptor defects the uterus was smaller than controls (50, 51).

The uterine tissues including the endometrium, epithelium stroma and myometrium all express high levels of ERα receptors as well as the fallopian tubes, cervix and vagina. Lower levels of ER β are also found (52). In mice models where the uteri do not exhibit ERα receptors, the uterus does not

demonstrate the same response to oestradiol treatment suggesting that ERα receptors are the more important receptor subtype (53). Oestradiol appears to have several effects on the uterus. It stimulates proliferative and antiapoptotic responses in the epithelium and also induces the expression of growth factors. IGF-1 is a key growth factor that mediates the effects of uterine growth response to oestradiol (54). There are oestrogen receptors located in the uterine artery wall and under the influence of oestrogen there has been shown to be a decrease is vascular resistance. The pulsatility index (PI) of the uterine artery decreases reflecting the increase in blood flow to the uterus and ovaries during the pubertal years (55). Cheuiche et al. examined the PI of 169 females between the ages of 5-16 years and found that PI in prepubertal girls was higher than in those in later pubertal stages. They demonstrated that the onset of puberty could be predicted by the reduction of PI and an increase in uterine volume (55, 56). The alteration in vascular resistance and blood flow to the uterus is also seen with exogenous oestradiol exposure which is important when considering uterine growth in the context of pubertal induction treatment (57).

There is more dramatic growth of the uterus from the age of approximately 8-10 years and following thelarche, uterine growth progresses throughout the pubertal years, driven by high circulating oestradiol concentrations (18, 19, 47) Without oestrogen, this stage of uterine growth fails to occur as seen in females with hypothalamic amenorrhoea and oestrogen deficiency during adolescence (58, 59). There is synergism with the somatotrophic axis as evidenced by the close relationship between peak growth velocity and maximum uterine volume change (19). There is ongoing correlation with pubertal stage and uterine development during these years. Hagen et al. demonstrated in 121 females progressing through spontaneous puberty that with increasing oestradiol levels the uterine

size in all dimensions increased with Tanner staging (46). There appears to be a peak in uterine development between Tanner stages 3-4 (15, 20, 47). Other papers have also found the same correlation with the pubertal stages. Many authors have found that uterine length, volume and fundal cervical AP ratio (FCR) are positively correlated with other parameters including chronological age, bone age, height and weight (17, 47, 60-62). The peak uterine growth during spontaneous puberty occurs between the ages of 8-14 and then growth plateaus from 14-20 years of age (18). Uterine growth steadily endures beyond that of menarche and full breast development, to conclude at approximately 20 years of age (20, 63, 64). Parity, and other pathologies for example fibroids, exert an enlarging effect on uterine size during adulthood but otherwise the uterine stays relatively constant in size until the age of 40 (16, 61, 65-67).

The occurrence of menses occurs approximately 2 years after thelarche in 90% of girls, with an average age of 13.75 years (29, 68). Initially menarche does not necessarily reflect ovulatory cycles as many early bleeds are as a consequence of oestradiol fluctuations rather than the synchronised hormonal patterns with ovulation (1).

There is significant variation in the post pubertal uterine size between individuals (18, 19). The determinants affecting this variation between individuals is unknown. In addition to the hormonal influence of oestrogen, progesterone and growth hormone other factors affecting uterine size may include variants in expression of candidate genes *HOX* and *Wnt* and prenatal exposure to maternal tobacco smoking (69).

IMAGING OF THE UTERUS IN SPONTANEOUS PUBERTY

The uterus and ovaries are amenable to size and morphological assessment using transabdominal ultrasound in females of all ages and ultrasound has been demonstrated to be accurate in assessing uterine and ovarian volume (70).

For the majority of young females requiring assessment, the transabdominal (TAUS) approach will be used. TAUS is observer dependent and assessment may also be compromised by variation in measurement protocols, practitioner experience, shadowing from bowel and bladder filling. This may make comparison between measurements and longitudinal evaluation a challenge. The transabdominal approach employs the bladder as an acoustic window and therefore bladder filling needs to be optimal, but if the bladder is over distended it can modify the uterine shape. The transvaginal (TVUS) approach may be used after sexual debut (65).

Several authors have used pelvic ultrasonography to assess uterine development during puberty and adulthood (18, 19, 21, 71, 72), but older studies are limited by sample size, restricted by small age ranges and being out of date in terms of ultrasound technology. More recent papers by Kelsey et al. and Gilligan et al. have provided nomograms per year in age for the different uterine parameters (18, 19). Gilligan et al. reviewed the retrospective ultrasound examinations of 889 patients between the ages of 0-20 years and Kelsey et al. examined a combination of MRI images and uterine volume data from previously published reports (19).

The literature is relatively homogenous with regards to conventional standardized uterine measurements that should be recorded. The standard ellipsoid formula to calculate uterine volume is uniform throughout the literature. There is, however,

some variation in practice. Some authors separate total uterine length into the cervix and body measurements, whereas others have favoured uterine length in its entirety. Using total uterine length circumvents the challenges faced in trying to differentiate between the two anatomically (16, 46) which potentially renders these measurements and their interpretation susceptible to interobserver discrepancy. In a similar vein, providing a ratio of uterine body to cervix based on length may be open to practitioner variation and therefore the ratio of Anterior-Posterior measurements have been used by some (15, 17, 21).

The prepubertal uterus is described as classically tubular in shape. The total length would be expected to be 25 - 40mm. The anterior-posterior diameter of the uterine body is approximately equal to that of the cervix in the prepubertal state, approximately 10mm with a FCR 1:1. The uterine volume is small between 1.5 - 3.5mls (16, 19, 46, 47, 64). The cervix predominates initially in the prepubertal state, but the converse is true in the mature uterus, and the uterine body increases in length and width relative to the cervix, giving rise to the mature pear shape. There is a consequential change in the cervical to body ratio to >1. During puberty, the total uterine total length increases to 50 - 80mm, the uterine body AP measurement increases to 30mm, and the transverse dimension increases to 30-50mm (3, 16, 45, 55, 65). The uterine volume in the later pubertal stages is approximately 25 – 78 mls (19, 20, 55). In later puberty the endometrium becomes ultrasonographically apparent and then fluctuates in thickness depending on the timing in the menstrual cycle (18).

Attention is usually focused on uterine length and the increase in uterine length relating with both age and pubertal stage is well established (17, 19, 47, 55). It has however been debated that uterine volume provides better correlation with

age and pubertal status in the pubertal years and therefore is perhaps a more useful parameter (19, 20, 64, 73).

Several ‘cut-offs’ which constitute optimal final adult uterine ‘maturity’ have been proposed but no consensus has been reached and there is a large spread of post pubertal size status. There have been such limits described for the distinction between prepubertal and pubertal uteri as this aids the diagnosis of precocious puberty (47, 64, 74, 75). The parameters suggested for the mature uterine length and volume varies significantly in the literature and therefore, it is difficult clinically to establish a clear delineation for the diagnosis of suboptimal uterine development and hypoplastic uterus. Some authors describe their normative values of maturity based on Tanner breast staging (20, 46, 47), whilst other authors use age criteria (17, 18, 64, 71, 76), and this makes comparisons a challenge. Importantly the fully mature uterine size may not be entirely captured as many authors assess size only until breast Tanner stage 5 is obtained or until the age of 15-16 whilst it is known that uterine growth continues after menarche for several years (46, 64). Therefore, this may not be truly representative of full adult development and may lead to misinterpretation of data and perhaps false reassurance.

Whilst in clinical practice ultrasonography has become the first line gold standard tool for uterine and ovarian assessment, Magnetic Resonance Imaging (MRI) is a complementary diagnostic tool which may add adjuvant clinical information regarding uterine morphology and pelvic anatomy. It has been possible to identify ovarian tissue with MRI when it has not been apparent using ultrasonography (77) and MRI has been cited to be more precise and accurate than US (19, 46, 77-80). Uterine size and volume utilizing MRI has also provided normative age-related reference ranges (19, 46). MRI and TVUS, whilst not subject to the patient

variables including bladder filling and body habitus, are not suitable initially or practical in the young female patient requiring regular review.

DELAYED PUBERTY AND PRIMARY AMENORRHOEA

Puberty will start in 95% of girls between the ages of 8.5 and 13 years. Breast budding (thelarche) is usually the first sign of puberty and will begin in most girls by 11.3 years. 95% of girls will have achieved breast Tanner stage 2 by 13. The occurrence of menses occurs approximately 2 years after thelarche with an average age of 13 years. By the age of 14.5 95% of girls will have periods (1). The exact timing can be affected by multiple factors including ethnicity and BMI.

Oestrogen deficiency is manifest in three ways depending on the stage in life when it occurs - delayed puberty, secondary amenorrhoea and menopause.

Primary amenorrhoea occurs in approximately 0.3% of females. Primary amenorrhoea with no secondary sexual characteristics by the age of 13 or in the presence of secondary sexual characteristics by 15 years of age is indicative of delayed puberty (1,2).

Some girls may already have a diagnosis with which pubertal development problems can be predicted, however for the majority pubertal delay may be first sign of an underlying issue triggering medical assessment.

Aberrations in the pubertal transition may be secondary to a spectrum of pathologies disrupting the HPO axis. If there is lack of both thelarche and menarche, this is indicative of complete early oestrogen deficiency. However, some may present with varying degrees of incomplete secondary sexual development suggesting an interruption at some point in the pubertal process.

When primary amenorrhoea occurs in isolation and other secondary sexual characteristics are present, this implies oestrogenisation and therefore alternative diagnoses should be considered. The cause may be anatomical rather than hormonal or alternatively it maybe secondary to acquired pathology occurring during puberty.

Hypoestrogenaemia concurring with anatomical anomalies has been described, however this scenario is incredibly rare. As late presentation and diagnosis are also common, this can create a clinical conundrum and a heterogeneous cohort (81).

Puberty is a significant milestone in a girl's life and its absence will not only have physical but also psychological sequela. Lack of 'development' and not keeping up with peers can be highly distressing and this should not be forgotten nor overlooked in the assessment and treatment.

CAUSES OF PRIMARY AMENORRHOEA

The aetiology of pubertal delay and primary amenorrhoea can be based on the presence or absence of secondary sexual characteristics (Table 1.1).

Table 1.1. Differential diagnosis for primary amenorrhoea based on the presence of secondary sexual characteristics

Absence of secondary sexual characteristics	Presence of secondary sexual characteristics
Constitutional delay	Uterine outlet obstruction
Chronic illness	Mayer-Rokitansky-Küster-Hauser
Hypothalamic amenorrhoea	Polycystic ovarian syndrome
Hypogonadotrophic hypogonadism	Hyperprolactinaemia and other endocrinopathies
Hypopituitarism	Complete Androgen Insensitivity Syndrome
Premature Ovarian Insufficiency	Pregnancy
Turner syndrome	
Swyer syndrome	

TURNER SYNDROME

Turner syndrome (TS), with partial or complete loss of the X chromosome has a prevalence of 1:2500 female live births. Different karyotypes of TS include monosomy X (45, X) (40-50%), isochromosome Xq (20%) and ring X chromosome (3-11%). Of those with mosaicism 45, X/ 46, XX accounts for 15-20%, 10% XY and 3% triple X (82, 83). The extent of the X chromosome loss usually dictates the severity of the phenotype and Turner syndrome may be associated with type 2 diabetes mellitus, neurocognitive issues, autoimmunity, cardiac pathology, ovarian dysgenesis, infertility, osteoporosis and short stature (83). Given this spectrum of clinical features, presentation may vary across the ages with some females diagnosed antenatally and others later in life.

The presence of TS renders the ovarian germ cell pool vulnerable to hastened atresia (6). Whilst initial oogenesis and follicular development is normal in those

with TS there is accelerated oocyte apoptosis and impaired folliculogenesis occurs during prenatal and early postnatal period (84). The ovarian follicles are replaced with fibrous tissue and somatic cells producing the characteristic 'streak ovaries' (85). The majority of women with TS, approximately 80%, will experience pubertal delay and primary amenorrhoea with the typical hypergonadotrophic hypoestrogenic hormonal picture at the expected time of pubertal onset approximately 11 years (86). TS accounts for approximately 10% of females presenting with primary amenorrhoea.

The karyotype can be predictive for the degree of ovarian preservation and reproductive phenotype with X chromosome dosage being an important factor (6, 81, 87). The integrity of the long arm of the X chromosome appears to be particularly important for reproductive function (88), with the Xq13-q26 being a particular critical region containing genes pertinent to ovarian development (89). Furthermore, the degree of meiosis chromosome pairing failure may affect the degree of ovarian function (90). The majority of women with TS who have a spontaneous puberty and pregnancy have mosaicism and it has been shown that at least 10% of euploid cells in those with mosaicism is predictive for spontaneous puberty (6, 87) as is the ultrasound detection of ovaries (62). It has been reported that those with mosaicism have a 50% chance of entering spontaneous puberty (85). Furthermore, the presence of a 45,X / 47,XXX karyotype or partial deletion of the short arm of the second X is associated with increased likelihood of preservation of ovarian function (58, 91, 92).

Whilst spontaneous puberty occurs most commonly, but not exclusively in those with mosaicism, the presence of monosomy does not preclude spontaneous conception (93, 94). This suggests that the karyotype may be different in the ovary, with gonadal mosaicism permitting oogenesis. It has been suggested that

mosaicism would be found in all fertile women with TS if extended genetic examinations were completed (92). It has been demonstrated that the cytogenetic analysis and level of mosaicism of the peripheral cells (lymphocytes, buccal cells or urine-derived cells) does not correlate to that found in the ovarian cells (95). The 46, XX content in the cells in the ovary predicts not only the degree of oocyte loss but also the adequate functioning capacity of the ovary. Fertility success depends on several factors not solely on an euploid oocyte. The supporting granulosa cells, required for follicular maturation and the control of meiosis I arrest in prophase, and the stromal tissue are all composite to reproductive integrity (95, 96). Therefore, if there is karyotype variation throughout ovarian tissue lines this may have implications for fertility success.

An alternative hypothesis for the ovarian function seen in some with 45, X is that other somatic alleles may provide compensation for the X chromosome deficiencies allowing preservation of ovarian function (94). The degree of ovarian preservation and function may also be influenced by X inactivation patterns and haploinsufficiency pseudoautosomal genes (97).

A widely held belief is that there is accelerated atresia in general in those with TS, however it appears that, to the contrary, ovarian loss encouragingly does not appear to occur at an accelerated rate in adults with TS who have spontaneous menarche and on-going ovarian function. In a longitudinal follow up AMH levels remained stable during the 5 year follow up (98), however further studies are required to provide absolute reassurance as to the maintained reproductive lifespan in this cohort of women with TS.

In addition to reproductive and fertility concerns, other stigmata of Turner syndrome may be present and require specialist investigation and monitoring

including growth optimisation and cardiac health (8). TS is related to congenital cardiac anomalies such as bicuspid aortic valve, coarctation of the aorta or renal abnormalities, increased prevalence of aortopathy and collagen defects and acquired cardiovascular pathology including aortic dilation and a predisposition to develop HTN (99, 100). The rate of aortic root dilatation and dissection is 32% and 1-2% respectively (101). Gonadoblastoma is a risk in approximately 10% for those with Y chromosome mosaicism, and therefore prophylactic gonadectomy is recommended (82) .

PREMATURE OVARIAN INSUFFICIENCY

Premature Ovarian Insufficiency (POI) with hypergonadotrophic hypogonadism is characterised by loss of ovarian function with amenorrhoea prior to the age of 40. The incidence of POI is quoted as 1% before the age of 40, 0.1% of women aged under 30 and 0.01% of women under 20 (102, 103). Therefore, primary amenorrhoea associated with premature ovarian insufficiency is rare, and secondary amenorrhoea is a far more frequent presentation.

Often the aetiology of POI remains unknown, however autoimmune, genetic and infective origins have been implicated. Autoimmune pathogenesis is implicated in approximately 30% of those with POI. Iatrogenic POI may be secondary to gonadotoxic chemotherapy, radiotherapy (7) or bilateral oophorectomy (103). There appears to be no single causative gene found for POI, with several complex genomic contributions affecting ovarian function identified (104). Autoimmunity is commonly associated with POI, including hypothyroidism (20%), adrenal insufficiency (10%) and other autoimmune conditions such as rheumatic arthritis, myasthenia gravis and system lupus erythematous.

The hormonal and reproductive function of the ovary is deficient in POI. The hypoestrogenic state may result in multiple health implications including cardiovascular, bone, psychological and reproductive sequelae. There may be fluctuation in ovarian function and therefore infertility and amenorrhoea may not be a permanent state for some women.

The diagnosis based on European Society of Human Reproduction and Embryology (ESHRE) guidelines, requires 4 months of amenorrhoea/oligomenorrhoea in combination with an FSH level of >25 IU/L on 2 occasions at least 4 weeks apart. AMH is not required for diagnostic purposes (103). Additional tests for aetiology include karyotype testing, fragile X premutation and autoantibodies including ovarian, thyroid and adrenal antibodies.

CONSTITUTIONAL DELAY OF GROWTH AND PUBERTY

Constitutional delay of growth and puberty (CDGP) is a common cause of pubertal delay with overall delay in the maturation of the HPO axis. It is more common in males than females, accounting for 65-73% of cases of delayed puberty in boys compared to 30-43% of cases in girls (105). There will be no other concerning features in the history and blood tests will reveal low gonadotrophins and oestradiol concentrations. All aspects of puberty will be delayed including growth velocity (3). CDGP is often familial with 79% having a positive family history and other relatives affected (106). The diagnosis is usually one of exclusion.

Whilst constitutional delay is considered a variant of normal, it can be clinically challenging to differentiate it from congenital hypogonadotrophic hypogonadism

(CHH). Diagnosis however may be aided by the distinct genetic profiles of CHH (5, 106) and more recently genes have been identified that underlie CDGP (107).

Treatment usually only involves reassurance and watchful waiting. For some oestrogen induction of puberty may be necessary (108).

CHRONIC ILLNESS

The mechanisms underpinning the pathogenesis of chronic illness and pubertal delay are multifactorial. The HPO axis regulates the coordinated release of FSH and LH but with persistent critical conditions the activity of the HPO axis is inhibited and hypogonadism is mediated by several factors including inflammatory cytokines and hypercortisolaemia (109). Any chronic pathology either physical or psychological may be causative. Cystic fibrosis, chronic cardiac conditions, coeliac disease, emotional and physical abuse are just a few examples.

HYPOTHALAMIC AMENORRHOEA

Hypothalamic function and GnRH pulsatility is sensitive to physical stressors such as extreme exercise, stress and calorific restriction leading to weight loss. The pathophysiology is thought to be mediated by leptin and depending on the timing of the insult, primary or secondary amenorrhoea may ensue. There is no particular threshold for the amount of exercise that will lead to amenorrhoea. The 'Female Athlete Triad' is often coined whereby there is amenorrhoea associated with decreased energy intake and low bone density (4). A BMI of at least 19kg/m^2 and a fat mass of 22% is thought to be critical for the onset of menses. Anorexia and bulimia, as well as other eating disorders such as orthorexia, may lead to amenorrhoea. Ultimately loss of hypothalamic drive will lead to

hypogonadotrophic hypoestrogenic state and 3% of primary amenorrhoea cases are attributed to hypothalamic amenorrhoea (4, 5).

Hypothalamic amenorrhoea should be a diagnosis of exclusion once all organic causes have been ruled out. Gonadotrophin levels may be normal/low with hypoestrogenic levels. Furthermore, blood tests may demonstrate hypercortisolaemia with hyperprolactinaemia and reduced IGF-1.

Although considered reversible if the underlying cause is eradicated for example weight restoration, clinically there is often a lag between removing the trigger and resumption of GnRH pulsatility and gonadotrophin release. Indeed, in some, despite near normal gonadotrophin levels being seen, GnRH pulsatility is never repaired fully and some may remain amenorrhoeic and hypoestrogenic permanently. Treatment is targeted at alleviating the underlying cause, however either temporarily or more long-term, hormonal replacement may be required (110).

HYPOGONADOTROPHIC HYPOGONADISM

Hypogonadotrophic hypogonadism (HH) be can be congenital or acquired with failure of the normal episodic secretion of gonadotrophic releasing hormone (GnRH). Congenital hypogonadotrophic hypogonadism (CHH) may be related to the production, secretion or functioning of GnRH at the genetic or molecular level. The incidence is 1–10 cases per 100 000 births (111).

Anosmia may be observed in approximately 50 % of patients with CHH. Kallman syndrome is a rare (1:120 000 females) condition whereby there is defective GnRH neuronal migration to the arcuate nucleus. It is associated with colour blindness and/or anosmia in combination with hypogonadism. Kallman syndrome

may be sporadic in nature but may also have X linked/autosomal dominant hereditary. One gene implicated is *KAL1*, which is essential for neuronal development.

Whilst CHH may occur in isolation, it may also be part of a more complex medical syndrome. Primary amenorrhoea may be one feature of rare clinical spectrum syndromes including Prader – Willi syndrome, CHARGE syndrome or Dandy Walker syndrome

In some (10-20%) with CHH reversal may occur therefore suggesting a degree of plasticity in the GnRH pathways. Whilst recovery is more common in males, resumption may occur in females and once oestrogen therapy has achieved adequate pubertal and bone development, many advocate a trial of stopping hormonal support to see if natural hormonal production resumption occurs (112).

There is complex genetic heterozygosity underlying CHH with mutations identified in over 40 genes responsible for GnRH neuronal development and migration as well as secretion and functioning of the GnRH. Most genetic defects are sporadic however they may be associated with various modes of inheritance. Genetic testing can be offered to patients with whole exome testing or directed panel sequencing, with gene mutations being identified in up to 50% of patients (111). Whilst there may be overlap with the genotype and the phenotypic presentation, finding mutations associated with CHH will allow prompt diagnosis, early distinction from CDGA and timely initiation of treatment.

Physical destruction or compression of the hypothalamic - pituitary axis may cause loss of GnRH/FSH and LH production. Hypothalamic and/or pituitary function may be compromised or completely lost as a result of the mass effect of intracranial lesions such as craniopharyngiomas. Furthermore, the treatment of

these tumours (surgical resection or adjuvant irradiation) can exacerbate the trauma to the area. Following radiotherapy, the gonadotrophic depletion may gradually develop several years after the initial insult. The hypothalamus and/or the pituitary may also be damaged secondary to head trauma or infections such as Human immunodeficiency virus (HIV) or Tuberculosis.

HYPOPITUITARISM

Global pituitary dysfunction with loss of the production and/or secretion of the pituitary hormones will lead to multiple comorbidities including hypogonadism. This may be congenital in origin in the case of rare genetic causes such as septo-optic dysplasia or may be acquired secondary to tumours including pituitary adenoma, trauma, infection and inflammation. There will be other clinical and biochemical evidence of hypopituitarism.

MULLERIAN DUCT ANOMALY

In the presence of a normal hormonal milieu and development of secondary sexual characteristics, primary amenorrhoea in isolation may suggest anatomical causes.

Uterine anomalies, also known as Mullerian duct anomalies, represent a spectrum of malformations seen in the female reproductive tract due to erroneous embryogenesis. The prevalence of uterine anomalies is approximately 5.5% in the general population. Despite their embryological origin, many are not detected until later in life due to an incidental finding or with the manifestation of their clinical sequela (9).

The Mullerian ducts undergo a series of complex transformations between approximately the 6-11th weeks of gestation to form the uterus and upper two

thirds of the vagina. The external genital and lower portion of the vagina develop from the urogenital sinus. Uterine/vaginal anomalies may ensue secondary to problems with vertical or lateral duct fusion or defective resorption of the septum. There may even be complete agenesis.

Gynaecological and obstetric presentations may include primary amenorrhoea, irregular menses, dysmenorrhoea, endometrioses, pelvic pain, infertility, obstetric complications or sexual problems. Due to their shared embryological origins, uterine anomalies are also associated with renal anomalies.

Mayer-Rokitansky-Küster-Hauser syndrome (MRKH) which affects 1 in 4500 is complete uterine agenesis. Due to an intact functional HPO axis, the classical presentation will be with primary amenorrhoea, normal gonadotrophins, presence of secondary characteristics and typically no cyclical pain. Less commonly, a rudimentary uterine horn with functional endometrium may persist. In this scenario the associated cyclical pain may confuse and delay the diagnosis. 10-15% of cases of primary amenorrhoea are thought to be caused by MRKH.

Whilst most cases are sporadic in nature there may be more rarely an autosomal dominant pattern. There are several candidate gene and genetic variants that have been associated with MRKH and Mullerian duct formation including *Wnt4*, *LHX1, HNF1B*, *TBX6*, *GREB1L* and *PAX8* (113).

Outlet obstruction may be as a result of different anatomical variations including imperforate hymen, transverse vaginal septum (high, medium or low) or vaginal/cervical agenesis. Imperforate hymen is the most common (1:1000), whilst transverse vaginal septum has an incidence of 1:80,000. Presentation maybe with primary amenorrhoea in combination with cyclical pain due to the accumulation of blood leading to haematocolpos and hematometra. Clinical

examination may reveal the visible distinctive ‘blueish’ bulge of an imperforate hymen but may not detect any abnormality if the obstruction is high. Imaging in the form of high-resolution 3D ultrasound (US) or MRI is key to aid diagnosis, to locate the level of obstruction and any associated anomalies such as renal anomaly.

Treatment may render the primary amenorrhoea completely reversible in the example of surgical management of imperforate hymen/transverse septum.

POLYCYSTIC OVARIAN SYNDROME

Polycystic ovarian syndrome (PCOS) is associated with increased LH production, hyperandrogenaemia and disrupted folliculogenesis. PCOS is usually associated with secondary amenorrhoea but may, more rarely, present with primary amenorrhoea. In adolescents, ultrasound diagnosis is not recommended due to the high incidence of polycystic morphology in this age group. Diagnosis is based on the combination of amenorrhoea and hyperandrogenaemia (clinically and biochemically) (10).

HYPERPROLACTINAEMIA AND OTHER ENDOCRINOPATHIES

Elevated prolactin levels exert negative feedback at the level of the hypothalamus/pituitary with subsequent loss of FSH/LH secretion. This may be secondary to prolactinoma or medications. Symptoms and signs described may be associated with the space occupying lesion itself such as headache and visual disturbance or may be secondary to the raised prolactin for example galactorrhoea.

Other endocrinopathies may interfere directly with pituitary function. Hypothyroidism leads to increased TSH levels which in turn stimulates both

prolactin and FT4 release. Insulin is also known to mediate the HPO axis at multiple points and therefore Type 1 Diabetes Mellitus may also be associated with pubertal delay. Hyperandrogenaemia with excess testosterone levels may be secondary to androgen secreting tumours, late onset Congenital Adrenal Hyperplasia and Cushing's syndrome and may lead to amenorrhoea.

DIFFERENCES OF SEX DEVELOPMENT

Differences of sex development (DSD) is a term encompassing many diagnoses where the 'development of chromosomal, gonadal and anatomic sex is atypical' (11).

The gonads remain undifferentiated until the 7th week of gestation. The Y chromosome houses the Sex Determining Region Y SRY gene which is responsible for the differentiation of the gonad into a testis. In turn, the testes produce both Anti Mullerian Hormone (AMH) and testosterone leading to the regression of the Mullerian ducts, persistence of the Wolffian structures and development of male external genitalia.

Primary amenorrhoea due to DSD may be secondary to either hormonal dysfunction or atypical anatomical development.

Swyer syndrome is a rare DSD affecting approximately 1 in 80,000 people. It is also known as complete XY gonadal dysgenesis. People with Swyer syndrome will have an XY karyotype but due to the gonadal dysgenesis and lack of AMH the Mullerian structures will persist and a uterus and upper 2/3 of vagina will develop. External genitalia will be of female phenotype. Multiple different gene mutations have been implicated in Swyer syndrome include SRY gene and the

MAP3K1. Pubertal delay and primary amenorrhoea will manifest due to lack of hormonal drive and hypergonadotrophic/ hypoestrogenic picture will be seen.

Androgen Insensitivity syndrome (AIS) is another XY DSD secondary to an androgen receptor gene mutation. Its incidence is 2 to 5 per 100,000 and it has an X linked recessive inheritance pattern, however, may result from de novo mutations. 5% of cases of primary amenorrhoea are accounted for by AIS. Due to the presence of functioning testes there is regression of the Mullerian structures, but lack of receptor receptivity renders the testosterone inactive and external female genitalia develops. Sparse axillary and pubic hair and a short blind ending vagina are characteristic. AIS may be complete or partial. With partial AIS there will be varying degrees of testosterone responsiveness and therefore virilisation, and those with partial AIS may present with atypical genitalia at birth. In contrast to Swyer syndrome, due to the aromatisation of the high levels of testosterone to oestradiol in the peripheral tissues, breast development and secondary sexual characteristics will develop but primary amenorrhoea will occur due to the absence of a uterus.

PUBERTY INDUCTION

For the vast majority of girls, the journey through puberty occurs spontaneously but those with early onset hypogonadism present with pubertal delay and will require induction of puberty with exogenous oestrogen. Spontaneous puberty starts with breast budding aged approximately 9-11 years and culminates in menarche aged between 12-13 years (29, 114); induction of puberty aims to mirror this as closely as possible.

There is no single consensus with regards to the protocol for oestrogen replacement in puberty induction and variation in clinical practice is common.

Many groups have assessed puberty induction protocols (82, 115-120). Consideration needs to be given to the age of initiation, the type and dose of oestrogen, the incremental tempo and later, the introduction of progestogen. Most evidence has been collected in females with Turner syndrome (TS). The most recent guidelines in TS suggest low dose initiation of transdermal oestrogen treatment between 11-12 years of age with slow dose titration to an adult dose over 2-4 years (82, 120). Some suggest a starting dose of 10% of an adult dose and increased 1.5-2.0-fold increments every 6 months (5, 120, 121). For those who present later, current opinion suggests a higher starting dose with faster dose escalation (5, 115, 122) as there are often competing physical, physiological and social needs.

Many inductions of puberty protocols suggest a fixed dosing regimen (5, 119, 123) which has also been examined in the literature (124), whereas others suggest dosing calculations based on body surface area (BSA) or weight (117, 125). There is no paper detailing the titration of dose based on hormonal and secondary sexual development outcome measures. A fixed dosing regime is felt to be simpler without the necessary adjustment at each visit which may be arguably more complicated for the patient. Labarta et al. examined two protocols using 17β oral oestradiol of either 5–15 mg/kg per day for 2 years or as a fixed dose of 0.2 mg daily during the first year followed by 0.5 mg daily for the second year. The fixed dosing regimen produced faster advancement to Tanner stage 4.

Progesterone should be considered when breakthrough bleeding occurs but should not be initiated unless uterine and breast maturity have been achieved (117).

Several endpoints may be used to assess the efficacy of treatment and to monitor the pace of induced puberty including the hormonal profile of E2 and gonadotrophins, uterine dimensions, body shape, height increment, bone density and breast development. Clinically oestradiol concentrations are measured routinely by immunoassay assessment. In early stages of pubertal induction treatment, E2 concentrations are usually below the limit of detection for this routine E2 immunoassays, which limits their use in this clinical context (126), however it has been suggested that if the oestradiol level is above the 40pmol/L lower limit of detection after the starting dose in 12-14 year olds then this should be considered excessive dosing (9). The oestradiol level should increase with each dose increment but remain below 180 pmol/L during treatment and reach an adult dosing level of approximately 350pmol/L at treatment completion (127).

Treatment should be individually tailored with titration of dose depending on the trajectory of development and careful monitoring throughout. Despite seemingly adequate oestrogen exposure many users do not fulfil uterine and breast maturity compared to peers who undergo spontaneous puberty.

A long term prospective needs to be taken, as adequate hormone replacement will need to be maintained until the natural age of menopause to ensure optimal bone mineralization and cardiovascular health. The implications of insufficient oestradiol exposure during the pubertal years will translate far beyond the adolescent years and therefore a standardised evidenced based approach is mandatory.

EXOGENOUS OESTROGEN FOR PUBERTAL INDUCTION

There is divergence across the world in opinion, which has changed over time regarding choice of oestrogen protocol. In the UK, transdermal (TD) patches/oral

17β-oestradiol are favoured over oral ethinylestradiol (EE) or conjugated equine oestrogen (CEE) preparations or the combined oral contraceptive pill (COCP). Females requiring pubertal induction, for example those with TS, may have underlying cardiovascular comorbidities with a predisposition to hypertension (HTN), tachycardia and aortopathy. Therefore, choice of hormone replacement is imperative to prevent additive metabolic risk. The type of oestradiol may also be an important determinant in uterine and breast development and may have different metabolic outcomes. The natural 17β oestradiol, due to its more physiological resemblance, is recommended in the latest guidelines (82, 120).

CEE is favoured in the United States, (128) however, CEE is not recommended in the UK due to its composition of multiple oestrogenic components with non-human androgens and progestogens and its adverse metabolic profile (129). Anxiety pertaining to comprised development of secondary sexual characteristics should preclude the use of Combined Oral Contraceptive Pill (COCP) for pubertal induction. Whilst it is commonly used for hormone replacement maintenance, there are concerns that the provision of progestogen too early could negatively affect uterine and breast growth, that the dose of oestradiol is too high initially, and unless the COCP is used back to back there will be fluctuation in oestrogen concentration.

Oral oestrogens may be either micronized 17β oestradiol or synthetic versions such as ethinylestradiol (EE). Oral preparations undergo extensive first pass metabolism in the liver, which has several implications. Firstly, its metabolism to its less biologically potent counterpart estrone necessitates higher original doses to be given and secondly there is altered hepatic production of procoagulation factors, sex hormone binding factors, triglycerides and inflammation. Oral preparations may also affect the somatotrophic axis with alterations and lowering

IGF-1 concentrations (130, 131). Furthermore, monitoring of treatment with oestradiol concentrations is not possible with EE, which is not ideal when wishing to monitor titration of dose.

Transdermal preparations may be in the form of either an adhesive matrix patch, releasing a steady daily amount of 17β-oestradiol, gel or spray. Transdermal E2 is the most physiological exogenous oestradiol preparation and by bypassing the liver can be used in lower doses and does not affect hepatic protein synthesis. Oestrogen gel/ spray may have the advantage over the patch in that it is 'invisible' but still allows flexibility with low starting dose without effects on liver metabolism. The gel formulation is not currently used clinically for the purposes of pubertal induction as it is difficult to ascertain and measure the small doses needed.

Many groups have examined the metabolic outcomes between different therapeutic preparations and TD appears to convey more beneficial effects on bone indices, lipid metabolism, clotting, cardiovascular profile and thromboembolic risks (124, 131-133). Nabhan et al. demonstrated faster bone accrual and greater final height with TD 17β-oestradiol compared to CEE (124). Langrish et al. (132) demonstrated that replacement with TD preparation compared to EE yielded lower systolic and blood pressures and caused less effect on the renin angiotensin system. Although oral oestradiol preparations are thought to exacerbate metabolic effects to a greater extent compared to transdermal oestradiol, to be more specific, this is more associated with EE and CEE.

Reassuringly, regarding body composition, markers of adiposity and metabolic profile, many authors have found no difference between 17β oestradiol in its oral and transdermal forms. Torres – Santiago and colleagues in 2012, (131)

completed a randomised controlled trial of 17β oestradiol TD 37.5μg versus oral 17β oestradiol 0.5mg daily in 40 women with Turners syndrome. The previous oestrogen therapy was discontinued for 6 weeks and the dose was titrated according to oestradiol serum concentrations to achieve that equivalent to spontaneous menstruating females. Both oral and TD groups achieved comparable 17β oestradiol concentrations, however, those receiving oral therapy had higher estrone and estrone sulphate concentrations. Similar effects were seen in body composition and lipid concentrations and bone indices. In a cohort of girls with TS, Reinehr et al. examined BMI during spontaneous and pubertal induction treatment and whilst there was no difference in BMI between the groups, BMI was also not affected by the dose of oestrogen or the route of administration (transdermal or oral) (44).

It has been demonstrated that compared to oral 17β oestradiol, transdermal oestradiol results in higher oestradiol concentrations at the same dosage, provides a more physiological oestrogen milieu and results in improved feminisation (121, 124, 129).

TRANSDERMAL OESTRADIOL PATCH PHARMACOKINETCS

Transdermal 17β oestradiol is the favoured choice of oestradiol currently for pubertal induction treatment. Matrix transdermal oestradiol patches can be cut into small pieces to establish lower doses to more effectively replicate the early stages of puberty and facilitate dose adjustment (9, 134). Patches can also be removed if necessary. Ankarberg-Lindgren and colleagues in 2001, with further work in 2014, demonstrated the ability to reproduce both the low dose and nocturnal oestradiol pattern seen in the early stages of puberty with the use of transdermal 17β-oestradiol patches in hypogonadal females (9, 134).

There is some variation in serum oestradiol concentration, despite the same dose, revealed by the study by Ankarberg-Lindgren et al. (134). Delivery of the drug and variation may be secondary to patch pharmacokinetics and pharmacodynamics. Dose is not thought to be affected by the cutting of the patch to manipulate the dose or storage temperature (135) and the variation in absorption appears to be less with the patch than oral oestradiol preparations (136). As an adhesive matrix patch, it is proposed to release a steady daily amount of 17β oestradiol but the results as to the reliability of this vary. Whilst some have demonstrated stable serum oestradiol levels throughout use (137, 138) other have suggested that there is fluctuation with peak and trough levels between dosing intervals (136) which may affect response.

VARIABILITY OF RESPONSE IN PUBERTY INDUCTION

There appears to be variability in physiological response between individuals undergoing puberty induction treatment (123, 124, 134, 139, 140). Over a 12-month period Nabhan et al., using a fixed dose protocol of 25mcg dose for 6 months then increased to 37.5mcg, showed variability in uterine and breast response. TD was also found to illicit a faster uterine response (124). Furthermore, in the paper by Yollin et al. optimal uterine growth was achieved in some individuals after 6 months and others required 2.5 years of treatment (141). There was significant variation in response to oestrogen treatment of a fixed dosing regimen by Labarta et al. (139) and also in the paper by Mart et al. (140) there was variation in the serum oestradiol levels despite equivalent dosing. It has not been fully understood what governs this but may be related to many factors including aetiology, severity of hypogonadism, treatment compliance, pharmacokinetics and oestrogen sensitivity at the receptor level (136, 142, 143).

PUBERTAL INDUCTION TREATMENT AND UTERINE DEVELOPMENT

Without oestrogen the uterus fails to grow and may be described as hypoplastic (58, 144), often being apparently absent on imaging (145, 146). Induction of puberty with exogenous oestradiol aims to mimic normal physiology but there is no conclusion as to how successful the process is in terms of uterine size (80, 115).

Uterine size has been relatively little used in routine clinical practice compared to Tanner staging charts and height, but it provides a more precise continuous parameter for individualised dosing of oestrogen which in turn prevents premature progesterone exposure and ensures adequate development.

Uterine maturity is comprised of not only an increase in length but also in total volume and thickening of the endometrium. The concept of a mature uterine configuration has been a factor often overlooked in many papers documenting and assessing only uterine length growth during pubertal induction. Different papers examining uterine development in pubertal induction use variations in their definition of uterine maturity in those with hypogonadism, making comparisons of papers a challenge. Some papers base definition solely on uterine length whilst other examine the ratio of uterine body and cervical AP measurements (124, 143, 147). Descriptions of the shape of the uterus are based on the uterine body/cervical AP ratio for example infantile/immature/juvenile if the cervix is larger than the body, cylindrical/transitional if the cervix and body are approximately the same and mature if the body is larger than the cervix (58, 116, 143, 147). Other authors have used a combination of length and shape to describe uterine development or uterine cross-sectional area (UXA)/volume (124,

147). A more recent paper by Obara-Moszynska et al. used uterine volume and FCR (123).

Longitudinal uterine size evaluation has been used to examine the efficacy of treatment with oestrogen in several papers (58, 115, 116, 123, 124, 140, 143, 144, 147-153). More papers now focus on uterine development as a primary outcome, previously less attention was placed on this parameter as height was the priority. Whilst it is unanimous that the uterus will be hypoplastic in the absence of any oestrogen exposure, research to date is minimal and discrepant with regards to how successfully adult uterine configuration is obtained in pubertal induction and the main factors influencing growth remain elusive. Whilst some studies have suggested that adult shape can be attained with exogenous oestrogen treatment (124, 140, 144, 148, 154, 155) others have reported to the contrary, with poor uterine development despite replacement (50, 58, 116, 123, 143, 149-151). Whilst some females undergoing pubertal induction may have a smaller uterine size than those going through natural puberty, it is difficult to establish when this is clinically important as there is no set cut off for defining the uterus as being 'too small'.

The importance to distinguish between uterine length and shape and to examine more than one uterine size parameter has been highlighted by several papers. Snajderova et al. (143) examined 27 women with TS between 18-42 years old who had undergone pubertal induction in a cross-sectional study using oral 17β oestradiol and they used 65 mm define mature uterine length in addition to the FCR. They found that only 37% of women attained a uterine length greater than 65 mm and that uterine length was affected by current daily oestrogen dose and age of menarche. 50% of women attained adult uterine shape based on ratio measurements, but interestingly not all these females had a length greater than

65mm and conversely some who had a mature length did not have adult shape. This highlights that the two uterine parameters are not always correlated and maybe affected by perhaps different elements. Paterson et al. (58), undertook a cross sectional study examining 20 women with TS ranging between 16-22 years, receiving daily ethinylestradiol doses of 20mcg or more and demonstrated that only 50% of females developed a mature uterine shape despite an average length of 63mm. Obara-Moszynska et al. examined 40 females with TS undergoing pubertal induction, and whilst FCR was no different to that an age-matched post pubertal group, the uterine volume was significantly smaller (123).

Numerous parameters may affect uterine responsiveness in those undergoing pubertal induction treatment. This may pertain to the choice of oestrogen preparation, dose, initiation age, duration of therapy, underlying pathology, use of growth hormone, window of opportunity and progesterone exposure. These factors have all been implicated when exploring suboptimal development. It is however problematic to compare papers due to the heterogeneity in methodology, outcome measures, participants and many review now outdated treatment protocols. Lack of concrete conclusions and ambiguity makes it a challenge when providing care for women with hypogonadism.

OESTROGEN PREPARATION AND UTERINE DEVELOPMENT

The oestrogen preparation may be a key factor as to the success of the development of secondary sexual characteristics in pubertal induction.

In a prospective randomised controlled trial (RCT) Nabhan et al. 12 females with TS aged between 11.3-17.1 years of age, treated with GH prior were treated with either TD 17β oestradiol or CEE treatment and the differences were examined after 1 year. There was superiority of TD in terms of bone indices, uterine growth

and breast development. No differences were seen in lipid profiles or IGF-1 concentrations between the two preparations. There were significant increases in uterine length and volume in the TD group compared to the CEE treated group (length 4.13 vs 1.98, volume 22.2 vs 4.0 p = 0.02) and 66% vs 0% in the TD and CEE respectively, developed a mature uterine total length >65mm (124). Bakalov also demonstrated worse uterine outcomes following treatment with COCP (150).

Piippo et al. demonstrated advantageous effects of gel percutaneous formulations on uterine development. In the prospective open multicentre study over 5 years, 23 females with TS aged between 10.7-17.7 years were treated with incremental doses of oestradiol gel (0.1mg in the first year followed by 0.2mg in the second, 0.5mg in the third year, 1mg in the fourth year and 1.5mg in the fifth year). Results demonstrated an increase in serum oestradiol levels from 22.2pmol/L to 162.2pmol/L at the end of treatment with a parallel decline in gonadotrophins. There was both an increase in uterine length and volume reaching a mean of 67mm and 31.5ml respectively (154).

In the study by Mart et al. uterine size was reviewed in 29 women with TS who had reached adult dosing of 17β oestradiol (either oral or TD) and 28 had been through pubertal induction with TD. 26/29 had Tanner stage 5 breast development and there was no difference between the uterine size compared to a control group (140).

Whilst it would appear that 17β oestradiol is the most beneficial oestrogen in terms of secondary sexual development it is not conclusive if a TD or oral delivery affects this further. Shah et at. examined pubertal induction using either TD or oral 17β oestradiol and found equivalent breast development to Tanner stage 3 after 6 months of treatment. Between the different preparations there was no

difference in body composition, change in height or inflammatory markers or lipid or liver profile. Although the authors did not measure uterine size, more girls using TD therapy achieved menarche during the study period (129). Kraus et al., in a small sample of 11 girls with TS randomised to either TD or oral 17β oestradiol, found that the increase in uterine parameters was similar in both groups and comparable to women without TS (155).

TIMING OF PUBERTAL INDUCTION AND DOSE OF OESTRADIOL ON UTERINE DEVELOPMENT

The timing and dose of first exposure to oestrogen has in the past been somewhat controversial, as the need for oestrogen replacement to attain adult sexual development needs to be carefully balanced with the necessity for optimal long bone growth.

Historically there were early concerns about starting age of oestrogen and worries over final height if started too early, however a delay in starting puberty induction treatment may have adverse consequences on peak bone accrual, cognitive function and in addition may result in negative body image with psychological effects (9). It was felt that excessive early oestrogen may precipitate premature epiphyseal closure with a detrimental effect on adult height. Indeed, in a study by Chernausek et al. 2000 (39), it was demonstrated that earlier initiation of oestrogen (12 rather than 15 years of age) using conjugated equine oestrogen (CEE) (0.3mg/d for 6 months increasing to 0.6mg/d), despite at least 1 year of prior GH use, led to a reduction in growth velocity with acceleration of bone maturity and consequential decrease in adult final height by 3.4cm.

High dose oestradiol has detrimental effects on final height but, as importantly, it also has a negative consequence on breast and uterine development. In the paper by Nabhan, a higher dose of TD E2 precipitated earlier break through bleeding in 4/6 of participants on TD preparation within the year study period and only 4/6 achieved uterine length maturity (124).

To circumvent these concerns and produce a more accurate replication of spontaneous puberty new strategies have been advocated. For those females, in whom oestrogen deficiency is predicted for example TS, GH, if required, can be started at a younger age with oestrogen in smaller doses following as young as 10 years old. Oestrogen dosage can be then titrated up accordingly (156, 157). In contrast to traditionally larger doses of oestradiol, this treatment strategy using, earlier more conservative oestradiol dosing may not compromise final height and may convey more beneficial effects on breast and uterine development, bone mineralization and cognitive function. Lower starting doses have been advocated by Ankarberg-Lindgren et al. showing that 0.08–0.12 μg/kg, corresponding to 3–7μg E2 patch can lead to appreciable changes in breast growth (134). As it is appreciated that there is some uterine growth even before thelarche driven by very low oestradiol concentrations, some have considered promoting growth with ultra-low oestradiol doses in the prepubertal doses, but the benefit of this protocol is not founded and not recommended (123, 158).

Kim et al. examined pubertal induction in 19 girls with TS aged between 13-17 years of age and the starting dose in 9 girls was 0.5mg and in 10 girls 1mg oral 17β oestradiol. In the 1 year follow up period, those who started with the higher dose had increased uterine development. Thereafter all participants had a gradual increase in dose to 2mg dose. At the final ultrasound assessment, a greater proportion of those who had started on the higher dose had a mature

uterus (defined by the authors as a uterine length > 65mm and FCR >1.1) 5/10 vs 2/9. This paper perhaps suggests that the starting dose may impact final uterine development (147).

In those who present late there are competing priorities of secondary sexual characteristics and bone health, as well as psychological considerations of keeping up with their peers. In these older age groups, the dose regimen is contentious and there is no single protocol although the start dose and tempo of dose change will need to be treated independently from early induction protocols (122). Low dose oestrogen with gradual dose increments may not be acceptable, and the protocol requires individual adjustment. It could be postulated small starting oestradiol doses for those with late onset induction of puberty may not be a sufficient start dose to promote the same uterine and breast changes as those seen in younger females. Mimicking normal physiology, early initiation of oestradiol is favoured but recently late onset pubertal induction has not been found to compromise final uterine size (115, 144, 150, 152, 156, 157). Gawlik et al. demonstrated that starting oestradiol treated either before 14 years of age or after the age of 14 had no impact on final uterine volume (115).

Whilst the temptation may be to increase the dose quickly in females who initiate treatment later, this should be avoided and despite time pressure at least 6-12 months oestrogen before the introduction of progestogen in all should be promoted. Too rapid replacement of oestrogen may adversely affect the cosmetic appearances of the breast (159).

The duration of therapy is also important with not too fast dose increments. In the study by Gawlick 73.5% of females eventually achieve menarche whilst on the

25mcg oestradiol patch (115), with an average of 1.5 years, suggesting that it is not only dose but also duration of treatment that is vital.

GROWTH HORMONE AND KARYOTYPE AND UTERINE DEVELOPMENT

Release of growth hormone (GH) from the pituitary is stimulated by oestradiol and it is thought to have a symbiotic relationship with oestradiol in growth and uterine development. It may therefore perhaps be expected that a relationship between growth and uterine size may exist if the two are directly interlinked, however both Paterson and Snajderova found no correlation with height and uterine length (58, 143).

The use of GH in TS, prior to oestrogen treatment, has been shown in some papers to have positive results on uterine development. Sampaola et al. reported a higher uterine volume in girls with TS who had received growth hormone than those who did not (160). Mondal et al. examined the uterine volume in 38 girls with TS who had received growth hormone for more than 1 year prior to the initiation of oestradiol treatment and found that the uterine volume was greater in those who had received growth hormone than those (n=35) who had not (161). The presumed beneficial effect of growth hormone is not unique to women with TS as Tsilchorozidou et al. suggested an additive effect of growth hormone and oestrogen deficiency on uterine size in those with GH deficiency and oestradiol replacement was not sufficient alone to induce optimal growth in this cohort (50). In contrast, in other studies, successful uterine development appears to be independent of either preceding or concurrent GH exposure in the TS cohort, conferring no additional beneficial effect (58, 124, 143, 147, 148, 150).

Some studies have suggested that there are variations in uterine potential based on the underlying TS karyotype. Mazzanti et al. studied longitudinal uterine

growth and found that women with mosaicism had greater uterine size compared to those with monosomy (62). Mondal et al. demonstrated that girls who had 45,X karyotype had smaller uterine volumes compared to the group with the other karyotypes (161). Doerr et al. and Bannink et al. (62, 116) also found that women with 45,X karyotype had poorer uterine length and volume measurements. This however is not conclusive as work by other authors do not concur, with no effect of karyotype on uterine development seen (58, 115, 123, 124, 140, 162).

WINDOW OF OPPORTUNITY AND PROGESTERONE

It has been commonly thought that an optimal window for pubertal development may exist, and that outside of this, deficient uterine and breast growth cannot be subsequently fully rectified and 'catch up' is not possible. The adolescent years are vital and if the uterus and or breast do not reach their genetically predetermined size during these years, then overall the size may be compromised. The determinants of this window are yet to be fully defined but is believed to be for both uterine and breast development, however the literature is not certain. Some have advocated replication of normal physiology with an early start to oestrogen therapy with a beneficial uterine response, whereas others have suggested though that timing of oestrogen replacement is not critical for uterine growth (115, 123, 144, 150). Perhaps the breast is more affected as Gawlik et al. demonstrated that although later oestradiol treatment did not affect uterine response, 3 older girls >14 at the start treatment had a resistance to oestradiol therapy with no change in breast response from Tanner stage 1 (115).

It may be that premature progesterone exposure could also hinder further uterine growth, as it has been seen that the plateau in uterine growth coincides to a similar time of spontaneous menarche with endogenous progesterone

production, approximately 14 years of age. Although growth of the uterus does occur thereafter, the pace is much slower (18, 67). Cleemann et al. completed a 5-year RCT in 20 women with TS who had completed pubertal induction treatment and had already switched to a sequential HRT regime. Participants received either an HRT regime containing 2mg or 4mg oral oestradiol. In the first 3 years there was a significant increase in uterine volume in those taking 4mg compared to 2mg suggesting that the uterus still has some capacity for growth after progesterone exposure and after the adolescent years (151).

It may be that the perceived suboptimal uterine size in those undergoing pubertal induction is because of a premature final assessment of uterine size, perhaps often at the time of break though bleeding and progesterone addition or after a defined period of time. Ongoing assessment should be recommended as it may be further uterine development occurs following completion of unopposed oestrogen treatment.

UNDERLYING PATHOLOGY AND UTERINE DEVELOPMENT

The underlying pathology leading to hypogonadism may be associated with integral uterine issues implicated for poor uterine development. Much work has focused on women with TS and it is theorised that uterine development is immature despite optimal endogenous or exogenous oestradiol exposure compared to those without TS (58, 62, 151).

Girls with TS in the prepubertal years appear to have smaller uterine size compared to prepubertal girls without TS even before the significant divergence in puberty between the two groups (161). It has been found that using ultrasensitive oestradiol levels, the circulating oestradiol levels are lower in girls with TS before puberty and the main pubertal years (163). Cleemann et al. found

that following pubertal induction treatment in 20 girls with TS that the uterine volume was significantly smaller than a control group (control vs TS: 51.6 ± 20.0 mls vs 30.5 ± 16.9 mls, P < 0.0005). In 4 of the 20 patients the uterine volume was less that the smallest volume seen in the controls of 18.7mls (151). Obara-Moszynska et al. in examined uterine size in 40 females with TS who had exogenous oestradiol treatment and compared to a post pubertal control group the mature uterine size in those with TS was significantly smaller (31.04 ± 11.78 mls vs 45.68 ± 12.51 mls, p<0.001(123)). Furthermore, in the paper by Kim et al. examining uterine size in 19 girls with TS, whilst the authors demonstrated that the starting dose of oestradiol treatment may be an important factor contributing for uterine growth, ultimately only 7/19 (36%) achieved optimal uterine maturity based on length and FCR at the end of oestradiol pubertal induction treatment (162).

Females with TS may have compounding intrinsic problems with uterine function including poor endometrial thickness, deficient uterine vascularity and altered vascular resistance, collagen deficiency, lack of X-linked genes regulating endometrial receptivity or poor epithelial integrity with lack of tight junctions (153, 164-169). Li et al. (169) examined the endometrial response by both ultrasound and histological examination to a standardised hormone replacement treatment and found that compared to women with idiopathic POI, women with TS had a poorer endometrial development in terms of both thickness and histological staging, suggesting an integral issue with the endometrium in women with TS. The authors hypothesised that this may be related to the concentration of steroid receptors present and their functioning. Furthermore, the use of HRT and current oestrogen dosing in those with POI and TS may affect uterine size, uterine blood flow and endometrial development (151, 153).

Whilst TS provides the focus of several papers examining uterine size, other authors have examined uterine development in females with alternative aetiologies leading to hypogonadism (50, 59, 147). Kim et al. (147) in a retrospective study examined changes in uterine length and AP FCR in 35 women; 19 women had TS, 10 had hypogonadotrophic hypogonadism and 6 had POI due to oncology treatment. Oral 17β oestradiol at varying starting doses was used for pubertal induction and the follow up period was for 2 years only. A uterine length of 65mm and FCR >1.1 was used to define maturity. A combination of these 2 parameters were used to categorise uterine development. Baseline uterine size was similar in the 3 groups and in all groups. POI secondary to cancer treatment (both chemotherapy +/- radiotherapy) was associated with poorer uterine development after 2 years of oestradiol treatment compared to those with TS or HH. Other confounding factors related to the cancer treatment such as total body irradiation may also affect uterine size.

Bumbuliene et al. explored uterine size in adolescents with functional hypothalamic amenorrhoea compared to a control group and found that oestrogen deficiency led to a smaller uterine size (59). Tsilchorozidou et al. examined uterine size in those with growth hormone deficiency, panhypopituitarism and hypogonadotrophic hypogonadism and found that despite oestrogen treatment and GH replacement the uterine size was smaller compared to a control group (50).

OESTROGEN RECEPTOR POLYMORPHISM

Gene polymorphisms may alter oestrogen receptor expression and function, which, in turn, may lead to individual physiological differences (170). Furthermore, variants in downstream signalling proteins may affect sensitivity to

oestrogen. This genotypic diversity has been linked to disease susceptibility and may also serve to modify interindividual biochemical and clinical response to therapeutic oestrogen (142, 170-172). The concept of oestrogen resistance at the receptor level as a cause of reduced response to treatment has been considered but not formally measured (115, 124).

UTERINE SIZE AND REPRODUCTIVE OUTCOME

Arguably, uterine growth could be the most important puberty outcome as suboptimal uterine size may be a contributing factor to reproductive outcome in the future (124) but the optimal uterine size for pregnancy is unknown. It has been hypothesised that the increased incidence of complications in pregnancy in adolescent females maybe related to incomplete uterine development and smaller uterine volumes (61, 173). The influence of uterine size has also examined in those undergoing IVF treatment (174-176). In women undergoing IVF treatment, uterine length has been shown to affect the chance of live birth with uterine lengths of <6cm being associated with increased risk of miscarriage (175). Egbase et al. found that the highest implantation and clinical pregnancy rates following embryo transfer were seen in women with a cavity length of 7-9 cm (174).

Women requiring exogenous oestrogen will be particularly vulnerable and therefore pubertal induction and optimising protocols to achieve uterine development is crucial. Uterine size affecting reproductive outcome has been seen in varying aetiologies requiring exogenous oestrogen treatment suggesting the uterus size is fundamental (63, 165, 177). Furthermore, risk may be further compounded by other aspects of uterine function specifically related by the underlying diagnosis (153, 164-166). This has been reported in the context of

women with TS whereby there may be several issues with uterine function unique to this diagnosis (165, 167, 169, 178). Overton et al. examined obstetric outcomes in women with pan hypopituitarism and reported increased rates of miscarriage, poor fetal growth and LSCS and suggested that this may related to uterine factors (177).

BREAST DEVELOPMENT IN PUBERTAL INDUCTION

During puberty the breast tissue develops slowly, taking approximately 4 years for full maturity. Initial breast growth is often asymmetrical but eventually usually resolves with no intervention required. In the context of pubertal induction, the average time required to achieve Tanner stage 2 is 6 months and Tanner stage 4 is approximately 2 years (82, 118), which is in line with spontaneous puberty but there is significant variation between individuals.

It is very challenging to review the literature and draw conclusions regarding the success of breast development with exogenous oestrogen given the heterogeneity in scientific literature. There are many influences affecting final breast maturity and shape satisfaction including genetic and epigenetic variations in spontaneous pubertal development (179). Furthermore, similarly, to the uterus, the breast tissue response to hormonal treatment may be dictated by multiple factors.

In women with TS, fundamental aspects associated to the diagnosis may affect in overall breast growth. Li et al. hypothesised that in those with TS, breast growth may be impacted due to altered steroid receptor concentrations in the target tissue (179). Doerr et al. found that in 18.6% of women with TS breast development did not mature beyond Tanner strange 3 despite optimal oestradiol replacement (149). Gawlick et al. demonstrated that after approximately 2 year

of treatment with exogenous oestradiol only 50% of participants reached Tanner B4 and that karyotype had no impact on breast development with exogenous oestradiol (115). In the study by Bannink, in those who underwent pubertal induction, breast development was eventually comparable to those with spontaneous puberty but with a 2-year lag. By the age of 19, 50% of those with TS reached B5 compared to 90% of women in the population data (116). Labarta et al. found that using a fixed or weight-based treatment protocol of oral 17β oestradiol regime only 42-65% of girls achieved Tanner stage 4 or more after 2 years of treatment (139). Other results are conflicting as Piippo et al. demonstrated that with oestradiol gel the rise in oestradiol concentrations correlated with the Tanner staging and 79% achieved Tanner stage 5 breast development and all reached Tanner stage 4 by the end of 5 years of treatment (154). In the study by Mart et al., in 29 women with TS who had been through pubertal induction using 17β oestradiol, 26/29 reached Tanner stage 5 (140). It appears that sufficient time of unopposed oestrogen is required.

Satisfaction with breast development has been found to significantly affect quality of life in women undergoing exogenous oestradiol treatment. (180-183). Idkowiak et al. conducted a survey of 74 women with TS using the BREAST-Q questionnaire. Those who had undergone pubertal induction reported lower breast satisfaction scores (180). In women with TS cited concerns have included mammary hypoplasia, asymmetry and tubular shape and whilst one paper found similar breast morphology to women without TS, the authors did report that in women with TS the thorax volume was more ‘bulky’ and the breast volumes were reduced (183).

Abnormal shape is a particular concern in those undergoing pubertal induction, but little is known about the causation. One hypothesis is hormonal imbalance

(24, 25) and in the context of pubertal induction, it could be extrapolated that the milieu produced by exogenous hormones may not replicate normal physiology sufficiently and the delicate balance between oestrogen and progesterone producing ductal forward growth and vertical lobulo-alveolar maturation respectively for optimal breast shape is not achieved. No oestrogen exposure will lead to breast hypoplasia but on the contrary, excessive starting dose and rapid escalation of oestradiol therapy may lead to fast paced horizontal growth which does not match the radial expansion leading to exaggerated anterior growth and protrusion of the areola. This may lead to a tubular shape with prominent nipple and areola and little peripheral supporting breast tissue (119). Tuberous breast is characterised by minimal fibrous supporting tissue with a smaller breast base, elevated inframammary fold, hypoplasia and glandular tissue herniation into the areola. The nipple and areola are not mature in appearance (23). Gawlik et al. suggested that those who start oestradiol later (after the age of 14) reach Tanner stage 4 faster than those who start treatment earlier (115). This may be related perhaps to the increased starting dose and faster incremental change in the author's regime as the majority of girls were over the age of 14. This fast pace of breast change was also seen in the study by Nabhan et al. (124) who used higher starting doses of oestrogen replacement in their study. Neither author commented on breast shape.

More aggressive replacement regimes using higher doses of oestrogen in the early phases of treatment, which is more commonly seen in those who present later and the trans female cohort may lead to overall compromised final breast development with premature termination of ductal branching and arrest of development at Tanner stage 3-4 (159).

The decision to add progestogen to the hormone replacement is based on several considerations including duration of unopposed oestradiol, the presence of breakthrough bleeding, uterine size and endometrial thickness. Recently it has been demonstrated that whilst some uterine growth still occurs in the presence of progestogen, albeit at a slower pace, this may not be the case for breast tissue (151). Therefore, breast development should also be satisfactory prior to a switching to a sequential hormone replacement regime. Ongoing maturation of the ducts is blocked by progesterone and therefore it could be hypothesised that if progesterone is added too early, prior to optimal development of the breast to Tanner stage 5, then abnormal shape and or suboptimal development may occur. In the paper by Gawlik et al. 73.5% of their cohort had breakthrough bleeding after an average of 1.5 years of oestrogen therapy and 94% of these patients had reached Tanner stage 3 only. If progesterone is then added as per the authors protocol, then progesterone may be added too early prior to full breast maturity (115). This was similar to the finding of Nabhan et al. (124).

Long term treatment and achieving the balance between prevention of endometrial hyperplasia, whilst providing ongoing unopposed oestrogen to facilitate breast growth, requires specialist services and regular uterine and breast surveillance. Late onset pubertal induction often favours higher starting oestradiol and faster tempo of dose change, which may in turn lead to earlier breakthrough bleeding necessitating progesterone addition. This leaves this cohort vulnerable to poor breast shape and growth.

Whilst high dose progesterone may be detrimental to final breast growth there is some evidence regarding the necessity of progesterone and its role in breast development. In puberty induction protocols the role of progesterone is not clear, and perhaps dose and timing are key factors. In women with spontaneous

puberty it is seen that breast growth continues post menarche and therefore perhaps progesterone exposure may be required in a cyclical fashion mimicking physiology (184). High dose progesterone is a treatment option for juvenile breast hypertrophy, blocking further growth, however in the context of gender dysphoria, it has been suggested that the addition of progesterone, in gender-affirming hormone therapy, is crucial for feminisation of breast tissue due to its effect on ductal branching (184, 185). There is variation in the breast volume during the menstrual cycle with an increase in volume in the luteal phase (186) and in the context of hormone replacement therapy, it has been shown that progesterone may play a role in the cyclical breast volume change suggesting that progesterone influences breast growth (187).

ASSESSMENT OF BREAST DEVELOPMENT

Breast development is a fundamental change during the pubertal years. For those who do not undergo spontaneous puberty, breast development can be promoted by the use of exogenous oestrogen therapy. Assessment of breast development is a vital clinical tool to ensure adequate oestradiol replacement and compliance with medication and should encompass breast growth as well as shape.

For the last 60 years the pictorial Tanner staging for breast development, first described in 1969, has been the mainstay method of assessing oestrogenisation through puberty but the subjective nature of a visualisation tool has many limitations (188). An objective method of continuous quantitative assessment of breast growth would have several benefits over Tanner staging including early detection of sensitivity to oestrogen and individualisation of treatment. Several breast assessment methods have been described in adults including direct breast

measurements, 2D photography, thermoplastic casts, ultrasound, MRI, water displacement and mammography (189-197).

The quantitative methods to date each have flaws associated which affects them being viable clinical methods (194). Furthermore, these techniques all rely on mature breast tissue being present, which is not applicable in those undergoing pubertal induction when breast tissue is minimal and immature, especially in the early stages where very subtle changes in breast borders are seen.

TANNER STAGING OF THE BREAST

To date, the clinical assessment of breast development is rudimentary and subjective, based on a pictorial scale first described by Tanner and Marshall in 1969 (188, 198).

The assessment of the breast tissue and nipple development is characterised into 5 stages. In stage 1 there is no glandular tissue. Stage 2 presents the first sign of puberty with the development of a breast bud (glandular tissues) which is palpable tissue under the areola, with enlargement of the areola diameter. In stage 3 there is tissue palpable outside the areola and further enlargement of the areola but there is no division in their contours. In stage 4 the areola becomes elevated above the breast contour to form 2 mounds and in stage 5 there is regression of the areola mound into the breast tissue with pigmentation and papilla formation of the areola and nipple protrusion (188).

With solely the use of a visualisation tool there is the room for significant inter observer variability, and the subjective nature has many limitations (188, 198). Tanner staging requires an experienced practitioner (188, 198, 199). Poor accuracy and reliability of Tanner staging occurs when completed by doctors with

minimal training (199), highlighting the need for thorough training and clinical experience. Furthermore, as the results are categorical, it takes time to determine rate of change and due to the subjective nature, there is also the difficulty in assessing and distinguishing glandular tissue from adipose tissue especially in those with higher BMI. This often leads to overestimation of Tanner breast staging.

OTHER MODALITIES TO EXAMINE THE BREAST

Progression of glandular tissue changes sees defined ultrasonographic morphologically appearances of the breast tissue based on the glandular, adipose and fibrous components which can be used to define maturity (192, 197). Breast development as assessed by ultrasound (US) has been shown to correlate well with Tanner staging (197). Breast volume, as assessed by US using the ellipsoid formula in conjunction with US morphologically appearances have been used in the assessment of precocious puberty (191).

Anthropometric data relies on standardised measurements between anatomical landmarks and formulae based on the female breast being half ellipse (190, 194, 200). The landmarks may be static i.e. between 2 landmarks but may also be dynamic where manipulation of the breast tissue is required. For standardised measurements patient positioning is also critical and needs to remain constant as breast shape, and hence anatomical landmarks, can vary depending on factors such as arm and back positioning and respiration. The intra and interobserver variability of anthropometric landmarking was reviewed by Issacs et al. (190) Whilst some measurements appeared reliable between observers some had significant error. Static measurements appeared to be most reliable

whilst the dynamic measurements were subject to significant interobserver variation.

Both MRI and CT have been used to assess breast volume in the surgical field (189, 201) and currently MRI is considered the current gold standard (202). Preoperative MRI and CT imaging volumetry was shown to correlate with the mastectomy specimen volume after resection (189, 201). MRI is considered the superior imaging modality in breast volumetric assessment in terms of accuracy and reproducibility (194, 202) however, it has drawbacks rendering it inappropriate for everyday practice including elements of time and cost. Furthermore, shape and size of the breast may be affected by the supine positioning required for MRI (193).

A thermoplastic cast can used to assess breast volume, whereby a negative 3D image of the breast is produced by placing the beast into thermoplastic material. The cast is then filled with water or sand to provide a volume (196). Often the volume using this technique is underestimated due to tissue compression (193). Archimedes' water displacement can be used whereby the breast tissue is placed into water and the breast volume is calculated based on the amount of water displaced (193).

3D BREAST IMAGING

The need for a quantitative assessment and in turn, the concept of breast imaging, has mostly evolved within the sphere of breast cosmetic and reconstructive surgery (200, 203, 204). Other areas of application are also being developed for example in the assessment of 3D breast volume change in pregnancy between 12 - 40 weeks' gestation (205).

Photographic systems can produce a topographic 3D image of the breast. It may be used to address both shape and volume and the technique has progressed using many different camera systems and software over time. Currently its application requires mature breast tissue and anatomical landmarking. Breast 3D imaging is a very attractive option as it provides a quick, non-invasive and objective assessment of the breast growth.

A standardised protocol of image capture is crucial to limit the source of error as the reproducibility of repeated imaging and measurements may be related to several factors (206). Anatomy and tissue definition may vary depending on patient positioning including head and arm placement and respiratory effort (202, 207, 208). This may lead to artefact and inaccurate assessment of true volume change.

Commercial software uses anatomical landmarks to produce a computer-generated estimation of the curved posterior boundary of the chest wall to produce a breast volume. The landmarks can either be placed manually (directly on patient or indirectly on the image) or automated by the software (209). In the study by O'Connell et al. (210) placement of markers by hand was deemed to have too much variation and the poor accuracy was impacting the reproducibility and therefore the authors developed a landmarking protocol for the purpose using a grid. They used their protocol to measure the volume of both in vivo breast tissue and phantom breast moulds. Whilst good levels of interobserver and intra observer reproducibility of volume assessment were demonstrated by O'Connell et al. using their bespoke landmarking protocol for mature breast tissue, the intraobserver variation obtained using the phantom breast moulds was less than with breast tissue (0.5% vs 3.8%). The authors conclude that this was due to the complexity of identifying the breast region of interest compared to the relative

ease of border definition in the phantom models. Other authors have also demonstrated an acceptable interobserver and intraobserver reproducibility of volume assessment calculated by 3D breast image using (161, 163, 172, 173)

Software automated placement of landmarks produces faster analysis, is more consistent and does not rely on operator experience. Automatic landmarking has been shown to be correlated with direct landmarking on the patient (209). Still, there are anticipated inaccuracies as software algorithms may not detect subtle changes in the breast borders and for absolute volume, assessment relies on computer analysis to interpolate the 'invisible' posterior boundary. To circumvent the limitations of automated software detecting the posterior chest wall, the 'subtraction method' to examine volume difference has been utilised to detect a difference in volume as compared to two absolute volumes in those with mature breast tissue (211).

Validity of 3D imaging has been investigated by comparing preoperative 3D breast image volume assessment with that of the actual excised tissue measured by water displacement (210, 212-214), and also implant volume by assessing differential volume between pre and post-surgical breast volumes (215) with a variation in volume by 2-12%. O'Connell et al. used 'phantom' breast moulds of differing volumes as measured by Archimedes' principle of water displacement. The average accuracy between the known volume of the moulds and the 3D volume assessment was an underestimation of 2.2% (210). 3D breast volumes have been shown to correlate to that calculated by MRI although agreement is more reliable at smaller volumes (193, 202). 3D volume assessment had also been seen to correlate well with surgeon experience and estimation (202).

3D BREAST IMAGING AND PUBERTAL INDUCTION

Whilst the literature is becoming more extensive as to the visualisation and clinical application of breast imaging, 3D imaging is a novel technique in the setting of pubertal induction. There is no current objective quantitative breast volume assessment utilised in this cohort and 3D volume assessment has not to date been used to examine breast development and longitudinal breast growth during pubertal induction. This group of patients has unique challenges and although lessons can be learnt from the surgical field, different considerations are required. With little or absent breast tissue in adolescents, landmarking is not a feasible option as the borders of the breast are yet to fully manifest. As seen, software, even at best, are subject to inaccuracies when landmarking mature breast tissues. Landmark placement is in turn required to produce the artificial posterior chest wall. Prior to oestradiol therapy there is minimal breast tissue and the automated systems for mapping the extremities of the breast tissue placement and volume assessment is anticipated to be inaccurate and, therefore limited in this cohort. Identifying landmarks either directly on the patient or on the image is rendered near impossible as there are often no borders to define and reproducibility of this technique is the largest barrier. It is therefore not a feasible technique to generate absolute volumes in this group based on the current protocols in the literature.

BREAST DEVELOPMENT AND ASSESSMENT IN TRANSGENDER GENDER-AFFIRMING HORMONE TREATMENT

In trans females undergoing gender-affirming hormone therapy, breast development and feminisation is a key feature for gender affirmation. In this cohort, the natural history of induced breast development is not well understood

or documented (216). There is no accurate monitoring tool, and consensus regarding the ideal oestrogen preparation and dosing for optimal breast development has not been established. The pictorial reference using Tanner staging may not be applicable in the transgender population and the other methods used to assess breast volume will be not feasible due to the problems of landmarking inadequate tissue borders.

There is no widely accepted method for monitoring breast growth in trans females. The Endocrine Society Clinical Practice Guideline on Gender Dysphoria states that breast growth in trans females can be 'partially' monitored by Tanner staging (216) which has been used in some studies to assess growth (217, 218). Historically, the use of hemi-circumference measurements (219), full breast circumference or bra cup size assessment (220-222) have also been utilised to assess breast growth with a hemi-circumference greater than 18mm corresponding to a B cup (223). These methods are limited as they usually rely on self-reporting and can be affected by several factors including respiratory effort (222). More recently 3D assessment using manual landmarking and software to produce a breast volume has been described in adult trans females (224) accepting the problems of landmarking with little breast tissue.

Oestrogen dosing and preparation protocols to optimise breast development in trans females vary and there are differences between adult and adolescent cohorts. Studies examining breast growth in the transgender population are limited and the natural history of breast development comparing different treatment regimens and variables affecting growth is sparse (184, 219-222, 224, 225). The heterogeneity of the literature regarding oestrogen preparation, age of treatment initiation, quantitative assessment of breast and follow up makes comparisons of the studies challenging.

Differences in breast development from cis females may be secondary to the anatomy of the cis male chest wall and also exposure to testosterone during endogenous puberty (27, 222, 223, 226). Volume assessment therefore may be particularly useful in the transgender patients as breast size may be underestimated due these differences in the cis male chest wall. In the cis female there is increased fat distribution and differences in the thorax shape (223), which soften the musculature contours. The breast development in trans females is also more lateral and lower on the chest wall compared to cis female, with a smaller areola diameter (224, 226). Furthermore, breast development in trans females may appear inadequate due to slower pace of fat accumulation and possibly, in adults, secondary to previous exposure to high testosterone concentrations, which inhibits breast development (222) consistent with endogenous testosterone affecting further mammogenesis during cis male puberty (27). These factors may cumulatively account for the subjective smaller growth of the breast and therefore volume assessment would be advantageous.

In contrast to pubertal induction treatment whereby transdermal 17β oestradiol is the preparation of choice, in trans females there may be superiority in using oral 17β oestradiol. In trans females an effect on liver metabolism may confer additional benefits due to the effect to increase SHBG which in turn reduces the bioavailability of testosterone (227).

Most studies examining breast development in trans females are in older adult patients who have completed endogenous puberty, are not using GnRHa treatment (225) and high supraphysiological starting doses of exogenous oestrogen in various preparations are used (219-221, 225). The study population by Dittrich et al. had a mean age of 38.3 years and the starting dose of oral 17β oestradiol used was 6mg which far exceeds the small doses in pubertal induction

(220). The study population of De Blok et al. had an average age in their study population of 28 years and the starting doses of the different preparations were consistent with adult HRT doses (222). A subsequent study by De Blok et al. had a study population of 26 years and started high dose oral 17β oestradiol (4-6mg daily) or transdermal preparation 50-100mcg changed twice per week. No obvious factors were identified to predict growth (224). Breast growth after 1 year was not affected by the oestrogen preparation, oestradiol concentration, BMI nor age initiation (222), however, breast growth in those using transdermal preparations was accelerated in the first 6 months but there was no difference after 1 year (222). Body mass index in trans females did not affect breast size which is to the contrary to that seen in cis females where an increased BMI is associated with increased breast volume (228).

In the adult population the onset of breast development usually occurs within 3-6 months of gender-affirming hormone treatment with maximal growth after 2-3 years (224, 229), although a more recent study suggests that the predominant growth occurs within the first 6 months and then plateaus, however this assessment was only made on breast circumference measurement (222). Fisher et al. demonstrated breast development to Tanner stage 3 after 2 years of treatment (217) suggesting that full growth may take several years which is consistent with cis females. With gender-affirming hormones there is variation in response in breast development. Overall the breast growth seems to be modest in the adult population, as in the study by Dittrich et al. only 5% achieved a cup size greater than B and 30% reported breast growth of less than A cup after 2 years of treatment (220). The study by De Blok et al. demonstrated that 47.8% had a bra cup size less than AAA after 1 year of treatment and 3.6% achieved a breast size greater than an A cup (222). After 3 years of treatment in the

subsequent study by Blok et al., breast growth in each breast was approximately 100cc (mls), which approximates to a cup size of less than A in 72% of women (224).

In contrast to the adult cohort, in the transgender adolescent population, who have had pubertal suppression, gender-affirming hormone therapy with exogenous oestradiol is initiated in the adolescent years and follows similar protocols as for hypogonadal females who have primary amenorrhoea and pubertal delay. This will aim to mimic the slow gradual process of puberty (216). Low dose regimes may optimise breast development (216). In contrast to the adult population, gender-affirming hormone started in the adolescent years appears to promote more promising breast development. This suggests that age of initiation may have an impact on breast growth, which has also been seen in females with pubertal delay (115, 116). In a study examining the initiation of oestrogen in the adolescent years (median age of 16) in 28 patients using oral 17β oestradiol, breast development started within 3 months of treatment which is consistent with the adult population however breast growth may continue for up to 3 years or more which is consistent with puberty in cis females (218). In addition to oestrogen replacement there has also been the suggestion that progesterone is vital in gender affirming therapy to enhance breast growth, due to its effect on ductal branching and the addition of progesterone could improve the breast and nipple size and shape (184). Furthermore, due to testosterones inhibitory effect on breast growth, antiandrogens may have a role in combination with oestradiol (227).

Overall, many trans females are unhappy with their breast development and up to 60-70% turn to surgical augmentation to obtain the breast development they desire (159, 220, 223, 227). Understanding factors associated with optimal breast

development may prevent dissatisfaction and optimise treatment with medical intervention alone.

OOCYTE DONATION PREGNANCY

Oocyte donation (OD) IVF is an option for parenthood. Oocyte donation may also be utilised for women with pathologies rendering them hypergonadotrophic with loss of ovarian function. Oocyte donation is now commonplace in the world of reproductive medicine and approximately 30,000 cycles are performed in Europe per year (230), with the first OD reported pregnancy in 1984 (231). Importantly, oocyte donation itself, regardless of the diagnosis and comorbidities of the female, is an independent risk for several outcomes (230, 232). A meta-analysis demonstrated that for singleton OD pregnancies the risk of HTH pathology was 13-39.3%, GDM 0-13%, LSCS 31.4 -85%, preterm birth 10 – 24.3%, and SGA 0-9.3% (230). This is important with regards to risk assessment and counselling. The causation for the risks associated with OD is likely to be multifactorial but suggestions put forward include advanced maternal age, nulliparity and immune maladaptation at the fetoplacental unit (232)

A meta-analysis of oocyte donation pregnancies reported an adjusted odds ratio of 2.30 for hypertensive disorders in OD pregnancy vs IVF using autologous eggs and singleton pregnancy. The risk is exacerbated further by multiple pregnancy (230). Hypertension in OD pregnancies is hypothesised to be placentally mediated as HTN has been linked with vascular aberrations in the endometrium/placental interface. It has been postulated that adverse immunological mediated reactions at the level of the placenta are stimulated by allogenic fetal antigens (233-235). Another mechanism implicated is that artificial endometrial preparation for OD cycles, without corpus luteum formation, prevents

some physiological adaptations seen in early pregnancy driven usually by the endogenous vasoactive and angiogenic hormones derived from the corpus luteum. This in turn leads to an increased risk of HTN and PET (236, 237).

The same placentally medicated mechanism may orchestrate the increased rates of postpartum haemorrhage, prematurity and low birth weight connected to oocyte donation. A meta-analysis of oocyte donation pregnancies reported an adjusted odds ratio of 1.75 for preterm delivery and 1.53 for low birth weight (230). However neonatal risk may be iatrogenic, a consequence of maternal sequela necessitating early delivery and the need for LSCS, the latter of which is also increased in OD pregnancy (230).

TURNER SYNDROME AND PREGNANCY

A proportion of women with Turner syndrome will retain some ovarian activity, with spontaneous menarche occurring in 5-20% of women and spontaneous pregnancy reported in 2-8% (238, 239). For those with ovarian insufficiency, oocyte donation (OD) IVF is a treatment option for conception.

Pregnancy in women with TS carries excess risks above the general population, irrespective of mode of conception (100, 164, 240-242). There is a culmination of risk factors including the diagnoses, comorbidities and pregnancy itself. Several hypotheses have been proposed as to the cause of increased obstetric risk; however, the extract aetiology is yet to be elucidated and is likely to be multifactorial. Obstetric concerns documented in the TS cohort, include an increased risk of miscarriage, preterm birth (<37 weeks), intrauterine growth retardation (IUGR) (less than 2500g or SGA <10th centile), hypertension pathology, caesarean section, aneuploidy, birth defects in the offspring and maternal mortality secondary to aortic dissection. The hypertensive disorders

include the constellation of pregnancy induced hypertension (PIH), pre-eclampsia (PET) and eclampsia and haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. Oocyte donation pregnancies are associated with elevated risks (230, 232, 233) which are compounded further by the TS phenotype. Women with TS who retain ovarian function and have a spontaneous pregnancy, still have increased obstetric risks including miscarriage (93, 164, 243, 244) and also risk passing on chromosomal anomalies to their offspring (92, 93, 243). The latter however is poorly quantified in the literature making accurate clinical counselling a challenge. Clinical care guidelines have been developed to ensure standardised preconception care and optimise antenatal and postnatal care (82, 120).

Much obstetric anxiety surrounds the cardiovascular risk profile associated TS. TS is associated with congenital cardiac anomalies such as bicuspid aortic valve, coarctation of the aorta or renal abnormalities, increased prevalence of aortopathy and collagen defects and acquired cardiovascular pathology including aortic dilation and an earlier predisposition to develop HTN (99, 100). Congenital cardiac pathology affects 20-50 % of women with TS and 50% of the adult TS population are affected by HTN (99, 245). Cardiovascular risk not only has implications for maternal morbidity and mortality but may also increase the risk of obstetric intervention and iatrogenic preterm delivery and low birthweight.

All women with TS considering pregnancy should have referral for pre-pregnancy cardiac counselling with recommended ongoing cardiac input throughout pregnancy. The 2024 published TS clinical care guidelines outlined that an echocardiogram should be completed within 2 years before conception with calculation of the ascending ASI. If the ASI >2.5cm/m2 or between 2.0-2.5cm/m2

with compounding cardiac pathology such as bicuspid aortic valve, coarctation of the aorta, HTN or elongation of the transverse aorta then the pregnancy should be avoided (120). Equally if there is a history of aortic dissection then pregnancy would be cautioned against. During pregnancy, as aortic dissection has occurred in women with normal pre-pregnancy cardiac status (240), an echocardiogram should occur at least once at approximately 20 weeks of gestation in the absence of any identified risk factors and every 4-8 weeks during pregnancy and up to 6 months postpartum if other risks present. During pregnancy HTN should be monitored regularly and be kept below 135/85 mmHg.

The risk of HTN in oocyte donation pregnancies in general is 13-40% and the pathogenesis is multifactorial and likely summative with TS (164). In women with TS, hypertensive problems complicated 35% of oocyte donation pregnancies (41/117) in the paper by Hagman et al. (240) and 37.8% of oocyte pregnancies in the paper by Chevalier et al. (100). Whilst many groups have found an increased risk on OD and TS compared to spontaneous pregnancies (100, 240, 246, 247), more recent papers have found comparable levels between the conception groups and lower rates than previous reports (241). The reduced rates seen in more recent papers may reflect the publication and adherence to clear consensus guidelines outlining the need for cardiac surveillance and optimisation (120).

Whilst there are multiple explanations for the increased risk of HTN in the TS OD pregnancies the above does not explain the mechanism in those with TS and spontaneous pregnancy. Other underlying issues related to TS have been hypothesised to compound the risk in all women with TS including endothelial, connective tissue and endometrial dysfunction (100, 248).

Aortic dissection is an apprehension to all health professionals caring for women with TS during pregnancy. The risk of maternal mortality secondary to aortic dissection is estimated to be approximately 100 times more likely in TS compared with women without TS (156). As aortic dissection occurs at an average age of 31.5 years, this is aligned with the reproductive years and provokes significant concern. Congenital cardiac anomalies such as bicuspid aortic valve and coarctation of the aorta and HTN predisposes to increased risk when combined with the haemodynamic changes during pregnancy and post-partum (156). Furthermore, women with TS may have collagen defects which also increase the risk of dissection. Aortic dissection has occurred in women with both spontaneous (164, 249) and OD conceived pregnancies (100, 240, 241).

The pregnancy rate per embryo transfer following OD in women with TS in the literature (17-52.3%) appears to be in line with OD in the general population with the better results in more recent papers likely highlighting the advent of superior assisted reproductive laboratory procedures and techniques (165, 166, 240, 247, 248, 250, 251).

Miscarriage in TS is greater compared to the general population regardless of mode of conception and is a consistent finding in all papers who report this outcome. Explanations for this may include oocyte factors (integral oocyte function and increased risk of aneuploidy) (243), those pertaining to the uterus or predisposition to thyroid autoimmunity (252) (73).

Genetic abnormality may affect the oocyte and stromal cells function affecting reproductive functioning in women with TS (95). Giles et al. reported miscarriage risk after PGT-A in women with TS who had undergone ovarian stimulation and found that there was still an increase in miscarriage rate (30.9%) compared to a

control group, despite PGT-A (250). This may be linked to the deficiencies in the integral function of the embryo despite euploidy. Interestingly OD did not mitigate this risk of miscarriage as the miscarriage rate in those with OD was still 23.8% (250). Bryman et al. also found the rate of miscarriage in those with TS having spontaneous pregnancy to be 45% but it was still 26% in those with oocyte donation pregnancies (164). Cadoret found that the miscarriage rate was higher in the OD group compared to the spontaneous conception group and an overall higher miscarriage risk in the TS OD group compared to the French population OD data (244), suggesting that OD does not circumvent miscarriage risk completely in TS. This implicates uterine factors.

In those with primary amenorrhoea hypoplastic uterine size and poor vascular supply may provide an explanation as to the mechanism of compromised reproductive. In those with secondary amenorrhoea and those with spontaneous conception it is theorised that uterine development in those with TS is suboptimal despite adequate endogenous or exogenous oestradiol exposure compared to those without TS. Paterson et al. (58) examined uterine development in females with TS with spontaneous puberty. Despite an assumed physiological oestrogen environment, only 7/10 females developed a mature heart shaped uterus.

There may also be several different fundamental mechanisms of the uterus that may be affected in females with TS including compromised endometrial and collagen function, vascular issues and integrity at the cellular level (153, 164-167). Furthermore, post pubertal HRT dosing may affect ongoing uterine size and blood flow (151, 153). This may provide explanation as to the increased miscarriage rate in TS, despite method of conception. In most papers reporting pregnancy outcome, data on uterine parameters is not available to explore this further.

Overall LSCS is greater in women in TS over the general population. Additional risks associated with the TS phenotype which increases LSCS rates may be related to short stature and feto-pelvic disproportion, patient choice, and expedited delivery due to obstetric concerns such as PET or SGA. LSCS may also be recommended and favoured by obstetricians, amid concerns that the haemodynamic alteration associated with labour may exacerbate underlying cardiovascular disease. Women with Turners syndrome tend to have a shorter stature and Hagman et el. found that those with TS had a shorter median height (161cm compared to 166cm in the reference group) (249). The increased rate of LSCS seen in women with TS may be related to short stature, which has been observed in both the papers by Hagman et al. and Bernard et al. (243, 249). This is a trend consistent in oocyte donation pregnancies; in the cohort examined by Hagman et al. those women who had a LSCS were shorter compared to those who achieved spontaneous delivery (152cm vs 159.5cm) (240). The 2024 clinical care guidelines for TS provide clear guidance on the cardiac consideration and delivery and LSCS is not indicated for all (120). A vaginal delivery can be considered in all women unless they have a history of aortic dissection, but attention should be paid to the second stage and be expedited where appropriate with an epidural anaesthesia (82). An individualised delivery plan should be made with a multidisciplinary team. Whilst LSCS may be the preferred mode of delivery for some patients and obstetricians, it should not be overlooked that LSCS also poses risks and is associated with haemodynamic changes (82).

Whilst some papers have found an increased neonatal risk in TS OD compared to spontaneous pregnancies, the recent paper by Cauldwell et al. found comparable rates between the two conceptions groups, and, following the same trend as HTN disorders, not significantly elevated above population data (241).

Offspring chromosomal abnormalities and birth defects have been demonstrated in women with TS and spontaneous pregnancies, and the risk of transmission of Turner Syndrome in the offspring is reported (90, 92, 93, 243, 253). In addition to the risk of TS transmission there has also been the suggestion that Trisomy 21 is also more common in the babies born (90), which is not attributable to increased maternal age. There is paucity in the data provided in the literature regarding karyotype analysis and there is often no systematic analysis of all female babies born making quantitative assessment difficult. Furthermore, given the lack of routine antenatal or postnatal screening cases, karyotype analysis has also been carried out later in life for other indications (93).

In the literature, the daughters with TS have had a variety of karyotypes recorded involving the heritability (90, 92, 93, 243). The latter is interesting as usually gonadectomy is recommended when the is Y chromosome material identified due to the risk of gonadoblastoma (83). The implicated gene is the oncogene TSPY, which is located on proximal Yp chromosome (254) and in some women this genetic material is not present. In some women with Y genetic material spontaneous menarche and pregnancy is achievable and been documented (164, 254). This fertility potential would not be the case if gonadectomy were performed, which makes counselling a challenge and an option could be a delayed procedure. Furthermore, genetic counselling is valuable as the precise genetic component of the Y chromosome present can alter the risk of gonadoblastoma and can provide individualised risk assessment.

Unplanned spontaneous pregnancies may occur (93, 94, 164, 241, 244, 255) which highlights the importance of contraceptive advice to prevent the need for termination of pregnancy and to allow for timely pre conception screening to prevent potential maternal morbidity in those who have high risk. Cauldwell et al.

reported the case of a woman who had an unplanned pregnancy with significant cardiac pathology (dilated aorta, bicuspid valve and coarctation repair) who underwent termination of pregnancy due to the significant risks of pregnancy (241).

TURNER SYNDROME AND SPONTANEOUS PREGNANCY

Spontaneous pregnancy in TS and pregnancy outcomes have been examined by several groups (90, 92-94, 164, 241, 243, 244, 249, 255-257) and the summary of outcomes is shown in Tables 1.2 and 1.3.

Bernard et al. examined 480 women with TS and 27 (5.6%) women had a total of 56 spontaneous pregnancies and 30 term deliveries. The findings from Birkebaek et al. recorded a spontaneous pregnancy rate of 7.6% of women (33/410) (92). Bryman et al. recorded 23 spontaneous pregnancies out of a cohort of 482 women with TS (4.7%) (164). Hadnott et al. undertook a retrospective data collection of 276 women with TS and found a lower rate of spontaneous conception (5/256 1.8%) compared to other papers (94). Doger et al. examined a cohort of 706 women with TS and identified 16 women who had a pregnancy with autologous oocytes (11 spontaneous pregnancy and 5 following IVF cycles), therefore the spontaneous pregnancy rate was 1.5%. In a Danish cohort of 410 women with TS, 31 (7.6%) achieved a spontaneous pregnancy and 2 had a pregnancy using autologous eggs with IVF (92). Nadesapillai described pregnancy outcomes in women with TS after they had undergone oocyte preservation. Nine women had 15 spontaneous pregnancies and 9 children were born. Two pregnancies ended in termination and 4 (25.5%) resulted in miscarriage (255)

Bernard et al. demonstrated that compared to the general population women with Turner syndrome and spontaneous pregnancy had an increased risk of miscarriage (30.8%), hypertensive disorders (pregnancy induced hypertension 13.3% and preeclampsia 6.75%) and necessity of lower segment caesarean section (LSCS) (46.7%) (243). Tarani examined 13 spontaneous pregnancies in 6 women and 8 live births. They reported a miscarriage rate of 6/13 46% (90). A paper by Bryman et al. reported both the outcomes of spontaneous and oocyte donation pregnancies in women with TS. Concurring with other papers, they found an increased rate of miscarriage (45%) and LSCS 63% (164). Retrospective data by Hagman et al., examining 205 spontaneous pregnancies in 115 women with Turner syndrome compared to matched controls, also identified an increased rate of preeclampsia (6.3% vs 3%) and LSCS delivery 35.6%. Miscarriage rates were not recorded (249). Hadnott et al. reported a LSCS rate of 57% but in contrast to the data by Bernard et al. (243) and Hagman et al. (249), hypertensive disease was not found to be increased and no case was identified, although the number of pregnancies examined was small. Doger et al, in 52 spontaneous pregnancies found a miscarriage rate of 67.3% The rate of LSCS was 47.1% and GDM 11.8% (256). Cadoret reported the outcomes of 23 spontaneous pregnancies and reported a miscarriage rate of 22.8% and LSCS rate of 45% and there were no cardiac complications (244). Cauldwell examined 73 spontaneous pregnancies in 62 women with TS. Most of the analysis was categorised based on karyotype rather than mode of conception. They found a rate of PET of 8.1% and a LSCS rate of 51.6% in spontaneous pregnancies (241). Calanchini examined the outcomes of spontaneous pregnancy in 21 women. 18 women had a live birth, 37 children were born and 46.7% of women experienced

miscarriage. PET complicated 11% of pregnancies, two women had GDM and LSCS was the mode of delivery in 70% of deliveries (93).

One case of aortic dissection has been reported in those with TS and spontaneous pregnancy (164, 240). Bryman et al. and Hagman et al. reported the same case of aortic dissection in 38-year-old women with TS (with Y chromosome fragment) at 7 months in her second spontaneous pregnancy. Both the mother and child survived. The findings of TS were unknown before the event and she was also found to have coarctation of the aorta (164, 249).

With regards to neonatal outcome and spontaneous pregnancy, there has been concern raised over the increased incidence of prematurity and low birthweight. The data by Bernard et al. reported that only 30 out of 52 (57.7%) pregnancies delivered at full term with a median birthweight of 3030g, but no further detail was provided (243). Hagman et al. reported an increased rate in preterm delivery (less than 37 weeks) 12.5% vs 5.2% and babies born with a birthweight <2500g 8.5 % vs 3.5% in those with TS compared to the general population (249). There was no case of prematurity of IUGR in the series by Hadnott et al. (94). Doger reported an IUGR rate of 5.9% and no cases of preterm delivery. There were no cases of prematurity or SGA in the series by Calanchini et al. (93)

Bernard et al. reported 2 cases of daughters born with TS (out of 17 female babies) with the karyotypes 45X,46Xr(X) and 47, X,der(X)t(X;Y)x2/46,X,der(X)t(X;Y). Other groups have recorded a range of birth anomalies without karyotype analysis (164, 249). Bryman reported congenital anomalies in the 5 children born including cerebral paresis, neuropsychological disorder, aortic coarctation, cleft lip and palate and congenital tumour however no karyotype analysis was completed. Doger reported karyotype analysis from 3

offspring and from 3 tested, 1 had trisomy 21, 1 had a normal karyotype and 1 had chromosome deletion affecting chromosome 18 (256). In the paper by Cadoret et al. the karyotype analysis was completed in 14 newborns and no chromosomal abnormality was reported (244). Calanchini reported a single case of TS vertical transmission. The daughter was diagnosed in adulthood following investigation for recurrent miscarriage and it was identified that she had the same karyotype as her mother (45, X/ 46, Xr) (93). Tarani reported the outcomes of 8 live births in 6 women and two (2/6 33%) daughters were born with TS. The fetal and maternal karyotype were concordant being 45, X/46, XrX and 46, XX, del (X)(p21) in the 2 cases respectively (90). Thirty seven percent of newborns had a karyotype analysis in the cohort presented by Birkebaek et al. thirty-two female babies were born and 5 (15.6%) female babies had karyotypes associated with TS (92). In the examination by Nadesapilliai 1 baby girl born had TS (255).

Table 1.2. Summary of pregnancy outcomes in women with TS and spontaneous pregnancy in the literature

	Total cohort Number	Number (%) of women with Spontaneous pregnancy	Number Pregnancies Number of children	Miscarriage %	HTN %	PET %	GDM %	Number Aortic dissection
Tarani, 1998		6	13 pregnancies 8 children	46%				
Birkebaek, 2002	410	31 (7.6%)	61 pregnancies 64 children					
Bryman, 2011	482	23 (4.7%)	82 pregnancies 37 children	45%	5%		5%	1
Hagman, 2011	115		205 pregnancies 208 children			6.3%		
Hadnott, 2011	276	5 (1.8%)	7 pregnancies 7 children		0	0	0	0
Doger, 2015	706	11 (1.5%)	52 pregnancies 17 children	67.3%			11.8%	0
Bernard, 2016	480	27 (5.6%)	52 pregnancies 30 children	30.8%	13.3%	6.7%	3.3%	0
Cadoret, 2018			23 pregnancies	22.8%				
Calanchini, 2020		21	37 children			11%		0
Cauldwell, 2022		62	73 pregnancies			8.1%		0
Nadesapillai, 2023		9	15 pregnancies 9 children	26.6%				

Table 1.3. Summary of pregnancy outcomes in women with TS and spontaneous pregnancy in the literature

	Number Pregnancies Number of children	LSCS %	Preterm <37 weeks	SGA	Chromosomal/ Congenital anomaly	% Female
Tarani, 1998	13 pregnancies 8 children				33%	75%
Birkebaek, 2002	61 pregnancies 64 children				15.6%	50%
Bryman, 2011	82 pregnancies 37 children	63%			5 birth defects 7%	
Hagman, 2011	205 pregnancies 208 children	35.6%	12.5%	8.5%	4.5% 1 Trisomy 21	84%
Hadnott. 2011	7 pregnancies 7 children	57.1%	0	0	0	
Doger, 2015	52 pregnancies 17 children	47.1%	0	5.9%	1 Trisomy 21 1 18q21.3 and q23.	70.6%
Bernard, 2016	52 pregnancies 30 children	46.7%			1 Trisomy 13 1 Trisomy 21 2/17 TS (11.7%)	56%
Cadoret, 2018	23 pregnancies	45%			0	
Calanchini, 2020	37 children	70%	0	0	1 TS 1/26 3.8%	72%
Cauldwell, 2022	73 pregnancies	51.6%				
Nadesapillai, 2023	15 pregnancies 9 children				1 TS	

TURNER SYNDROME AND OOCYTE DONATION PREGNANCY

Several papers have examined the feto-maternal outcomes of pregnancy after egg donation in women with TS and the summary of outcomes is shown in Tables 1.4 and 1.5 (93, 94, 100, 164, 240, 241, 244, 247, 248, 250, 251, 258).

Table 1.4. Summary of pregnancy outcomes in women with TS undergoing OD pregnancy

	Number (%) of women with pregnancy	Number Pregnancies Number of children	Miscarriage %	HTN pathology %	GDM %	Number Aortic dissection
Bodri, 2006	21	17 pregnancies 8 children	47%	62.5%		0
Bryman, 2011	30 (6.2%)	42 pregnancies 31 children	26%			0
Chevalier, 2011	93			37.8%	3.6%	2
Hadnott, 2011	5	6 pregnancies 7 children				0
Alvaro Mercadal, 2011	14	18 pregnancies 11 children	44%	50%		0
Hagman, 2013	106	122 pregnancies 131 children		35%	9.4%	1
Cadoret, 2018		62 pregnancies	37.7%	27.4%	11%	0
Andre, 2019		39 pregnancies 25 children	28.2%	28.2%	7.7%	0
Giles, 2020		80 pregnancies	23.8%			0
Calanchini, 2020	7	12 pregnancies 9 children	25%	0%	28.5%	0
Cauldwell, 2022		54 pregnancies		11.1%		2

Table 1.5. Summary of pregnancy outcomes in women with TS undergoing OD pregnancy

	Number Pregnancies Number of children	LSCS %	Preterm <37 weeks	SGA
Bodri, 2006	17 pregnancies 8 children	100%	50%	55%
Bryman, 2011	42 pregnancies 31 children	80%		
Chevalier, 2011	93 pregnancies	81.7%	38.3%	27.5%
Hadnott, 2011	6 pregnancies 7 children	100%	33%	57%
Alvaro Mercadal, 2011	18 pregnancies 11 children	80%	30%	40%
Hagman, 2013	122 pregnancies 131 children	82%	12.3%	17.6%
Cadoret, 2018	77 pregnancies	74%		24%
Giles, 2020	80 pregnancies	53%		8.7%
Calanchini, 2020	12 pregnancies 9 children	71.4%		
Cauldwell, 2022	54 pregnancies	88.8%		

Bodri et al. in 2006 examined 17 OD pregnancies in 21 women and 8 children were born. The results suggest that pregnancy rates are in line with other oocyte recipients, but the miscarriage rate was high 47% as was the rate of LSCS (100%) and HTN pathology 62.5% (247). Bryman et al. (164) reported outcome data for women with TS undergoing oocyte donation pregnancy and 30 women out of 482 (6.2%) achieved a pregnancy. The rate of miscarriage was 26%, significantly lower than that reported for the spontaneous pregnancies (45%). The rate of LSCS was 80% in those with OD. Chevalier et al. (100) reviewed pregnancy outcome after 20 weeks' gestation in 93 women with TS who underwent oocyte donation pregnancy. The rate of hypertensive pathology was 37.8% and GDM 3.6%. The LSCS rate was 81.7%. Hadnott et al. reported outcomes of 5 women with TS OD pregnancy resulting in 6 pregnancies and 7 live births. Compared to spontaneous pregnancies reported in the same paper, the rate of LSCS was higher (100%) and the rate of PET was 16.6% (94). A series by Hagman et al. 2013 (240) reviewed OD pregnancies in 106 women with TS between 1992 -2011. There was a total of 122 pregnancies, 131 children born, and obstetric data was reported for 117 pregnancies. The live birth rate per embryo transfer in women with TS was comparable to other OD cycles (33%). There was no data presented for early pregnancy complications, but the authors reported a rate of HTN 14.5% and PET 20.5% (35%) and this did not seem to be related to multiple pregnancy as in singleton pregnancies the rate of HTN pathology was 30.4%. The risk of GDM was 9.4% and the LSCS rate was 82%.

Alvaro Mercadal et al. in 2011 examined the outcomes following fertility treatment in 23 women with TS with a total of 49 cycles and 45 embryo transfers. The pregnancy rate per embryo transfer was 33%. The risk of early pregnancy loss was 44%. Fourteen women had a pregnancy and 9 a live birth. There was a total

of 18 pregnancies and 11 babies born. The rate of HTN complications was 50% and LSCS 80% (248). Andre et al. reported pregnancy outcomes in 73 patients who had a total of 151 embryo transfers. The clinical pregnancy rate per embryo transfer was 25.8% and the miscarriage rate was 28.2%. Twenty-five babies were born.

Giles et al. compared the outcomes of OD pregnancy vs the use of autologous oocytes and PGT-A. 70 women underwent a total of 165 OD cycles, 153 embryos transfers and there were 80 pregnancies. The clinical pregnancy rate per embryo transferred was 52.3% and the miscarriage rate was 23.8%. The LSCS rate was 53% (250). Cadoret et al. reviewed 62 OD pregnancies after 2009 and the miscarriage rate was 37.7%, which, in contrast to other papers, is higher than the rate they found in spontaneous pregnancy in women with TS (22.8%). The risk of HTN pathology was 27.4%, GDM 11% and LSCS rate 74% (244). Fourteen women had a total of 39 OD treatment cycles in the series by Calanchini et al. Seven women had 12 pregnancies with 7 live births and 9 babies were born. Three (25%) pregnancies ended in miscarriage and there were no cases of HTN pathology and 2/7 (28.5%) developed GDM. The rate of LSCS was 71.4% (93). Cauldwell reviewed 54 OD pregnancies in 45 women with TS and the rate of LSCS was 88.8% and PET 11.1% (241).

In the paper by Bodri et al. the risk neonatal outcomes were high with prematurity in 50% and SGA in 55% (247). Chevalier et al. demonstrated lower levels than Bodri but still an increased risk of preterm birth 38.3% and SGA 27.5% (100). Hadnott et al. reported an increased rate of prematurity and birthweight <2500g in those with OD compared to spontaneous pregnancies. Neonatal outcomes were more reassuring in the paper by Hagman et al. with a preterm birth rate and SAG rate of 12.3% and 17.6%, which was increased in multiple pregnancies

(240). Alvaro Mercadal et al. demonstrated a preterm birth and SGA rate of 30% and 40% (248), Giles et al. reported an SGA rate of 8.7% (250) and Cadoret et al. an SGA rate of 24% (244). Calanchini et al. reported the preterm birth rate of 14.2% (93).

Chevalier et al. reported 2 cases of maternal mortality secondary to aortic dissection (100). The first patient was 33 years of age, had aortic dilatation diagnosed in the second trimester, and then had aortic dissection at 38 weeks' gestation. The second case, also a 33-year-old woman died 7 days postpartum after delivery at 38 weeks for other obstetric indications. In the series by Hagman et al. there was one case of aortic dissection in a 28-year-old with a mosaic karyotype and a normal pre pregnancy cardiac assessment. She presented in the post-partum period and underwent successful surgical intervention (240). In the series presented by Cauldwell et al. documenting TS pregnancies between 2000-2020 over 16 different centres in the UK, two women had an aortic dissection. The first developed an aortic dissection at 18 weeks of gestation and survived, the second had an aortic dissection 2 days after a LSCS for HTN and died. Both women had 45, X karyotype and conceived via OD (241).

PREDICTION OF REPRODUCTIVE PHENOTYPE IN TURNER SYNDROME

Prediction as to reproductive phenotype in those with TS and with this, who may benefit from fertility preservation, is challenging. Which clinical markers of ovarian reserve and when to carry out the assessment is not clear cut. This is complicated further as clinical assessment is often carried out several years prior to the onset of natural puberty in an attempt to forecast reproductive function. In the absence of a single discriminative marker assessment should be based on pubertal staging, hormonal profile and karyotype.

The karyotype may serve as a useful predictive tool and indeed the karyotype is highly prognostic of ovarian function with monosomy being more susceptible to oocyte depletion (259) and follicles being most likely in those with mosaicism (260, 261). In the study by Lunding et al. spontaneous puberty was seen in only 7% with 45, X compared 88% of those with 45, X/46, XX. However, the occurrence of spontaneous puberty and pregnancy, the presence of ovarian follicles in OTC and successful ovarian stimulation has been completed in those with monosomy and therefore this should not preclude these females from fertility preservation consideration (93, 98, 261-263).

Signs of hormonal competence of the ovaries may also be used clinically. Borgström identified that ovarian follicles were present in 62% of those with spontaneous menarche and 58% of those with signs of spontaneous puberty (262). Evaluating menstrual cycle regularity is also affected by the maturing of the HPO axis and the physiological oligomenorrhoea commonly seen secondary to anovulatory cycles in the early years post menarche (264). Therefore, oligomenorrhoea cannot be used in the same predictive manner before impending POI as in the later years.

Hormonal markers of ovarian reserve and function have also been clinically used. In the early postnatal period, there may be an amplified FSH surge seen in females with TS reflecting the underlying early ovarian failure at the time of ‘mini puberty’ (86), however thereafter due to the quiescent state of the hypothalamic ovarian pituitary axis until the time of natural puberty, the clinical picture of ovarian failure with hypergonadotrophic hypogonadism will not be seen as the negative feedback system is not yet activated. In the prepubertal years gonadotrophins, oestradiol and inhibin B will all be low which is the same for those without TS (85). Therefore, in the prepubertal years gonadotrophins will not be useful clinical

markers of ovarian function or serve a prognostic role, in the same way as they are used in the post pubertal females (85).

Anti-Mullerian Hormone (AMH) has been suggested as an alternative surrogate marker of ovarian function as it is produced by the granulosa cells in the pre antral and antral follicles (265). In adults it is commonly used as a marker of ovarian reserve in the fertility clinical workup, however its role in indicating the exact time of oocyte depletion in adults is imprecise (266). In the series of 9 females with TS who underwent ovarian stimulation aged between 18-26 by Talaulikar et al. the AMH ranged between 3-21 pmol/L. The authors did not identify a correlation between AMH or antral follicle counts (AFC) and the oocyte yield suggesting that AMH may not be a useful marker in this clinical situation (100). Therefore caution should be exercised and decision making should not be based on a single marker.

In the paediatric prepubertal cohort, when gonadotrophins are not valuable diagnostically, AMH has been found to be a quantitative marker of the number of small antral folicles (79). The AMH levels reflect the antral follicles < 6mm (85). In those without TS, AMH rises in the postnatal period and then again between the ages 4-8 years but thereafter remains relativley stable during childhood and adolescence (98, 267). This suggests that AMH levels studied in childhood could predict the levels at the time of puberty and whilst there is a significant 15- fold variation of AMH levels between girls, levels will be maintained from childhood to adolescence (268). In the study by Lunding et al. longitudinal data in 120 females with TS revealed that an AMH <-2SD or <4 pmol/L in prepubertal girls was suggestive of absent pubertal development and an AMH of <5 pmol/L was indicative of imminent POI in adolescence. An AMH <3 pmol/L had both a sensitivity and specificity of 95% for POI in adolescence and adults (98).

Furthermore a detectable AMH in girls with TS is related to a 19 -fold increased chance of have spontaneous breast development and menarche compared to those with undetectable AMH (269).

Imaging the ovaries to identify ovarian follicles can be used and antral follicle count in the adult population prior to ovarian stimulation is routine practice (270). In the paediatric population a TAUS or MRI could be used, but these both have limitations (80, 271). Many of those with TS will not have ultrasonographically detectable ovaries (62) and the visualisation of ovaries or uterine volume have not proved useful markers for the prediction of spontaneous puberty (120).

Different algorithms have been proposed to aid decision making for when to intervene and fertility preservation treatment and for whom (85, 272, 273), but there is little data to validate the approaches. Serial AMH assessment has been suggested but this may be limited by the known natural fluctuation between measurements. Furthmore some have suggested measurements of AMH every 2-3 months (273) whereas others have advocated annual assessments (85) or every 2-3 years (274) highlighting the variation in practice. The exact trajectory in those who have had some degree of ovarian activity is difficult to predict.

FERTILITY PRESERVATION STRATEGIES IN TURNER SYNDROME

With the new modern era of reproductive technology there is heightened interest in the use of fertility preservation strategies in those with TS. This in turn has provided further insight and understanding into the unique reproductive profile associated with TS.

Strategies for fertility preservation in women with TS are important to provide the possibility of biological offspring in the future for some. The concept of fertility

preservation in those with TS was first aired in 2001 (275) and there have since been significant advancements in this field. Current options include ovarian stimulation with oocyte cryopreservation and Ovarian tissue Cryopreservation (OTC). Ovarian stimulation is reserved for those who are post pubertal and have ovarian activity and a menstrual cycle, whereas OTC can feasibly be carried out at any age. The burden of both the possibility of infertility and decision making regarding fertility preservation options and timings may have implications for both the patient and or the parents and therefore the wellbeing of the individual should be paramount (276).

It has previously been suggested that there may be increased aneuploidy rates in oocytes from women with TS (90, 164). Poor egg quality in combination with an already deficient egg number raises the questions as to whether sufficient quality eggs would be collected from ovarian stimulation or OCT and if these are justified procedures given the surgical and anaesthetic risks, coupled with the comorbidities associated with TS (263). Furthermore, fertility preservation should only be considered in those who have no contraindications to surgery or to a future pregnancy, although surrogacy could be considered for the latter.

For those who have compromised ovarian function and are post pubertal, ovarian stimulation (OS) and oocyte preservation is an option for women with TS and there have been several reports documented in the literature (255, 263, 272, 277-280). This has been completed in TS women of both adult (263) and adolescent ages (278, 280). Frozen thawed embryo transfer (FTET) is an established practice in the fertility sphere with success rates comparable to the use of fresh oocytes (281, 282). There is minimal data about any implications of TS on oocyte health in terms of vitrification, thaw survival and fertilisation. Ovarian

stimulation is associated with risks and thankfully the complications recorded have been minimal following ovarian stimulation (255, 263).

Results from non-TS individuals less than 35 years old cohorts, that could be extrapolated to those with TS in absence of sufficient data, suggests 10 to 20 oocytes are needed for one live birth, with the chance of live birth per oocyte cryopreserved approximately 6.5% (281, 283, 284). Given the concern regarding oocyte / stromal aneuploidy and increased risk of miscarriage, coupled with the perhaps already compromised ovarian reserve compared to those without TS, more oocytes are likely to be needed for a realistic chance of pregnancy. It is likely therefore that more than one cycle of treatment will be required.

Not all women with TS will have successful treatment with oocytes cryopreserved. In a cohort of 10 TS patients undergoing OS only 8 patients successfully cryopreserved oocytes (280) and in another cohort of 33 women only 88% had successful oocyte vitrification (255). The number of oocytes collected appears to be correlated with markers of ovarian reserve (AMH, AFC and FSH), and the karyotype (255). Brouillet et al. found the combination of low FSH (<5.9 IU/L), high AMH (>1.13 ng/ml) and the presence of >1% 46,XX cells was predictive for collecting at least 6 oocytes (285). Importantly though, successful egg yield following ovarian stimulation has been identified even when the parameters of egg reserve appear low (263, 272, 280) and also been successful in those with 45, X, (263) suggesting that karyotype alone should not be used in isolation to inform decision making, Patients have to be counselled that OS requires daily injections, regular monitoring of follicle development and transvaginal oocyte collection (273). Therefore, it is important to ensure that the patient has the psychological and physical maturity to undergo this procedure.

Ovarian tissue cryopreservation (OTC) since 2002 to date has only been practiced in an experimental context in the context of TS (260-262, 280, 286). OTC in TS follows the now established practice of OTC in females facing gonadotoxic chemotherapy and iatrogenic POI (287). OTC is gaining more traction for non-malignant indications (288). In other patient groups OTC has proved to be a successful intervention as live births, following spontaneous conception or IVF, after re-grafting of transplanted tissue harvested in both adulthood and childhood tissue have been reported (289, 290). Furthermore, there has been some restoration of endocrine function following tissue grafting in those who had OTC for oncology indications, although the duration of this window varies (291). In a large series examining ovarian tissue reimplantation in 285 women 88.7% regained menses and the spontaneous conception rate was 40% (292). Whilst the data outside the context of TS is increasing, the data in TS patients is extremely limited and therefore the outcomes uncertain.

Ovarian tissue cryopreservation may provide the only opportunity for those who are prepubertal (293) and allow invitro oocyte maturation (IVM) or transplantation of the ovarian tissue in the future (277, 286, 294). Some case reports have reported OTC in TS as young as 3 years of age (295). OTC is more invasive as it requires a laparoscopy for both harvesting and re-grafting and therefore carries surgical and anaesthetic risks. An international consensus suggested that 'O*TC should be offered to young females with TS, but in a safe and controlled research setting first, with proper counselling and informed consent procedures, before offering this procedure in routine care'* (260). Surgical and anaesthetic risks need to be carefully balanced and removal of ovarian tissue may theoretically compromise ovarian function further by removing ovarian tissue (85). AMH

appears to be the best predictive marker, with the strongest correlation to the presence of follicles in ovarian cortex tissue (120).

The success of fertility restoration following ovarian tissues cryopreservation and auto transplantation is dependent on the quantity and quality of oocytes collected with sufficient numbers of morphologically normal ovarian follicles within the tissue excised. In contrast to females facing oncology treatment whereby a high yield of healthy ovarian follicles would be expected, this cannot be presumed in those with TS. In a study of 57 girls with TS aged between 8-19.8 years found that ovarian follicles were only identified in 15 (26%). Whilst the highest rate was seen in those with mosaicism, 3 girls with follicles identified had monosomy (262). In another series, follicles were only found in those with mosaicism and not in those with 45, X (95). In the study by Mamsen et al. 15 females with TS between the ages of 5-22 underwent OCT. Follicles were found in 9/15 (60%) of the biopsies. Eight patients had a mosaic karyotype and 1 had monosomy. All patients who had follicles had a detectable AMH and/ or FSH <10 IU/L (261). There is no reliable methodology to determine who will have follicles present in the ovarian tissue, but the probability is greater in those with a mosaic karyotype, have been through spontaneous puberty, and have a detectable AMH with normal FSH levels (261, 271).

Overall, there appears to be a smaller density of follicles in the ovarian tissue of those with TS compared to those without TS (271). Given the attrition rate of follicles during the thaw process and in the days following re-grafting procedure (approx. 50% of follicles undergo ischaemia), this loss may have implications for the reproductive success of the transplant if the starting numbers of follicles are low, as would be expected in those with TS (296-298).

The genetic component and health of all ovarian cell lines may play a fundamental part as to the long-term reproductive success and is a consideration for those undergoing OS and OTC. A euploid oocyte does not guarantee normal oocyte function as genetic aberration may not only affect the oocyte itself, but also the follicular stromal cells required for successful oocyte function (299). Aneuploidy within the granulosa cells and the stroma cells may potentially affect fertility procedures such as oocyte warming and fertilisation, in addition to folliculogenesis and ovulation (95). Cytogenetic analysis using fluorescence in situ hybridization (FISH) of oocytes from small ovarian follicles collected from ovarian tissue biopsy demonstrated that whilst most oocytes (91%) were euploid there were higher aneuploidy rates found in granulosa cells (95, 271), which may still have implications for the full functioning of the oocyte. The implications of granulosa aneuploidy have been explored in a murine model suggesting that folliculogenesis can still take place (298).

Quality may also be determined by examining ovarian follicle morphology. In the series of OTC by Mamsen et al. despite follicles being identified there was a high rate of abnormal follicle morphology (261), seen in 6 out of 9 patients. This included abnormal oocyte shape with vacuoles present and incomplete and misshapen granulosa cell layers. Abnormal morphology was also identified in the study by Nadesapillai et al. (271). There is paucity of data about the success of fertility procedures in those with TS compared to those without TS. In a retrospective study in those without TS, a total of 1,283 vitrified oocytes were warmed for 128 autologous IVF treatment cycles and the live birth rate per embryo thawed was 6.4% (282). This is in comparison to the 3% reported by Strypstein et al, although this is based on a single case report (272). Furthermore, albeit based on a single patient, the success of IVM was less in TS, with higher

rates of degeneration and lower rates of oocyte maturation compared to controls (261).

In a Swedish cohort study of 100 patients with TS counselled regarding fertility perseveration OTC was planned for 73% and OS for 10% (280). Whilst the uptake appears to be high in this study, it is difficult to draw conclusions as to the success of use of the tissue thereafter. There is paucity of data relating to the long-term use of vitrified oocytes or ovarian tissue which probably relates to the fact that the patients have not yet reached the age for use yet. Out of 73 patients in one study, only 2 have undergone reimplantation of ovarian tissue and endocrine function was not regained (280). In a retrospective cohort of 33 women with TS who underwent OS between 2010 and 2021 no one yet has returned to use the vitrified oocytes (255).

The first pregnancies following both the use of vitrified autologous oocytes and auto transplantation of ovarian tissue in those with TS have recently been reported (272, 300). The first live birth following the use of vitrified oocytes was in a woman with 45, X/ 46, XX who underwent OS aged 25. Two cycles of ovarian stimulation yielded 29 mature metaphase II oocytes. At the time of oocyte use, 23 oocytes survived the thaw process and following intracytoplasmic sperm injection (ICSI), 13 oocytes fertilised, and 3 blastocysts were created and 2 were euploid (272). An uneventful pregnancy occurred following the frozen thawed embryo transfer (FTET). In the context of ovarian tissue in 2011 a case report documented the successful live birth following allografting of ovarian tissue from one monozygotic twin to another who both had TS mosaicism (301). The first case report of a clinical pregnancy after auto transplantation of ovarian tissue in a patient with TS was reported in 2023. The patient had mosaicism and underwent OTC at the age of 15. At the time she had entered spontaneous

puberty and undergone menarche and had a low FSH (1.9 U/L,) but an undetectable AMH. The patient had re-grafting of the harvested ovarian tissue at the age of 24 when her cycles were irregular and her FSH was raised. Thereafter her cycles regularity resumed, and she had a positive pregnancy test after spontaneous follicular development with cycle monitoring and induced ovulation however the pregnancy ended in a missed miscarriage (300).

Currently fertility perseveration in those with TS is not clinically established. Firstly, it is difficult to predict the rate of ovarian loss and if the oocytes and follicles will be functional and secondly there is no consensus regarding the protocol for decision making. In adult women with TS who have spontaneous menarche and menstrual cycles, AMH levels have been shown to remain stable over time suggesting the ongoing hormonal and reproductive capacity of the ovary (98) and therefore, fertility preservation would not be indicated in this group. Furthermore, a high (33%) proportion of women in one paper who have undergone ovarian stimulation have subsequently fallen pregnant without needing to use their stored tissue which questions the need for the procedure in the first place, including in one woman who did not have any oocytes cryopreserved after stimulation (255). Another consideration is the age at which ovarian stimulation is completed because it has been shown that females under the age of 20 have a higher rate of oocyte aneuploidy because of meiotic nondisjunction events (302). In contrast, in those whereby no follicles would be predicted to be present treatment should not be offered and any false optimism given. Caution needs to be exercised as in some of those with TS, despite markers suggestive of the presence of ovarian follicles, for some there were no follicles present in the retrieved tissue or the follicles were abnormal. This highlights the importance of careful clinical workup

and counselling. A timely and fully informed consultation is clinically required as this needs individualised decision making (262, 276).

TURNER SYNDROME AND GENETIC SCREENING

For those with TS and pregnancy following spontaneous conception, or after treatment with autologous oocytes the decision and options for genetic screening needs to be discussed.

The motivation for this is to try and mitigate some degree of the increased miscarriage risk perhaps related to increased oocyte aneuploidy in TS (90, 164) and also reduce the risk of TS vertical transmission (90, 92, 93, 243, 253). Options for testing may include preimplantation genetic testing for aneuploidy (PGT-A) or antenatal testing.

Karyotype analysis has been carried out on oocytes after ovarian stimulation and following OTC. Reassuring results, with euploid oocytes have been found (271, 277). For PGT-A sufficient numbers of embryos for testing are required, which may be a limitation in those with TS and necessitate multiple stimulation cycles. Certainly, in 56 patients with mosaic TS with a mean age of 38, undergoing OS for PGT-A, 35/65 (52%) cycles did not result in embryo transfer due to poor stimulation response or no euploid embryo to transfer (250). Only 28.9% of embryos analysed were euploid (250). In another series 68 women with TS underwent OS and 213 blastocysts were biopsied for PGT-A (303). The aneuploidy rate was higher and the cumulative live birth rates lower in women with TS in women compared to the control group (303).

PREMATURE OVARIAN INSUFFICIENCY AND PREGNANCY

There is paucity of data on reproductive outcomes in women with idiopathic POI. Much work has been extrapolated from the general OD cohort for OD pregnancy outcome. Regarding spontaneous conception, work has focused on the clinical predictors for resumption of ovarian activity, but little has been reported on the reproductive outcomes in those with spontaneous conception.

In women with idiopathic POI, there may be fluctuation in ovarian activity with spontaneous pregnancy rates quoted at approximately 5-10% (103). Bidet et al. examined 385 women with POI and demonstrated evidence of resumption of ovarian activity in 24% (304, 305). Other papers have quoted rates of spontaneous ovulation in 11-46% of women with POI measured by both hormonal and US parameters (306). Different markers may be used to suggest resumption of ovarian activity, including hormonal levels such as normalisation of FSH or detectable AMH levels, follicle growth on US, the presence of menses, or indeed conception (307). The study by Bidet et al. demonstrated that resumption of menstrual cycle was the most common initial presentation in the majority (79%) of those with intermittent ovarian activity, with occurrence most commonly in the first year after diagnosis (304). There appears to be not one indicative factor or test to provide guidance as to who is more likely to have restoration of ovarian function. Bidet et al. demonstrated that positive predictive factors for resumption of ovarian activity were familial POI, secondary amenorrhoea, follicles present on US, and oestradiol, FSH and inhibin B level (304).

The prediction as to the timing of ovarian restoration and the duration of this window is challenging. A further paper by Bachelot et al. examined 507 women and found that 23% of patients had evidence of some resumption of ovarian

function within 48 months of diagnosis and cessation of restored ovarian activity was predicted by initial high FSH levels, older age of diagnosis and high DHEA levels. After 1 year of follow up, 75% of those who had resumption continued to demonstrate evidence of ovarian activity (305).

Many different groups have examined varying treatment regimens hoping to increase the potential of ovarian activity, spontaneous conception or assisted conception with autologous oocytes in women with POI. To date no treatment has been proved effective (305, 308, 309).

Given this unpredictable nature regarding the resumption of ovarian activity, for those wishing to prevent an unplanned pregnancy, contraception advice is clinically necessary. HRT is not contraceptive and therefore in women not wishing to conceive, contraceptive advice should be given.

The age at which ovarian function ceases has been hypothesised to have an impact on reproductive outcome. It has been previously suggested that the trajectory of reduced fertility with the decline in both oocyte number and quality preceding the onset menopause may also apply to those with premature menopause (310). This led to the speculation that a diminished ovarian reserve may be a contributing factor to miscarriage risk in younger women (311). More recent papers have provided extra reassurance (312), suggesting that there is follicle depletion but not ‘reproductive ageing’ per se as pregnancy in women who were subsequently diagnosed with POI had a similar rate of miscarriage in the preceding years to the general population (313). Chine et al. examined 538 women less than 35 years of age undergoing IVF treatment and found that a lower ovarian reserve, based on AMH levels and AFC, was not associated with an increased risk of miscarriage if conception occurred (312). Anti-Mullerian

hormone levels are a marker of quantitative rather than qualitative assessment of oocytes. Whilst it is used to predict response to ovarian stimulation in the context of IVF (314), it is not a marker of oocyte quality and therefore not a predictor of miscarriage in those with POI or in the general population (315). Maternal age remains the largest contributing factor to miscarriage risk in POI. The incidence of thyroid autoimmunity is however higher in POI which may be an independent risk factor for miscarriage (103).

There is paucity of data examining the obstetric outcomes of women with idiopathic POI who conceive spontaneously on HRT. Bidet et al. examined 21 spontaneous pregnancies in 15 women with idiopathic POI. There were 4 (19%) miscarriages reported and 1/13 (7.6%) singleton births was preterm. Two case report by Calik-Ksepka et al. and Gu et al. both detail a single spontaneous pregnancy in women with POI and both pregnancies were without complication with delivery at term (316, 317).

FERTILITY AND PREGNANCY OUTCOMES IN WOMEN WITH TURNER SYNDROME COMPARED TO WOMEN WITH PREMATURE OVARIAN INSUFFICIENCY

Fertility and pregnancy outcomes in women with TS are usually compared to either data from spontaneous pregnancies in the general population or all donor conception pregnancies. Oocyte donation reference data is limited by intrinsic heterogeneity as there are many indications for OD treatment including previous oncology treatment, increased maternal age and women with naturally declining ovarian reserve. Women with idiopathic POI provide a better comparison group and there have been no direct comparisons of fertility and OD pregnancy outcome between those with TS and POI. Women with TS and idiopathic POI

share several predisposing factors which may affect the outcome of fertility treatment and makes them more susceptible to pregnancy complications. The incidence of thyroid autoimmunity is higher in both conditions which is associated with increased miscarriage risk (252, 318) and uterine factors may contribute to increased risks in pregnancy (153, 165, 166, 169, 175).

CONCLUSION

In summary, there are disparate conclusions drawn in the literature and significant variation in opinion as to what is considered best practice. Greater exploration into oestrogen replacement in individuals with hypogonadism will allow more understanding into the natural history of pubertal induction and factors affecting oestrogen sensitivity. The aim is to provide more bespoke, evidenced based therapeutic interventions and monitoring tools to provide greater clarity on reproductive endpoints and the potential reproductive sequelae.

CHAPTER 2

METHODOLOGY AND STATISTICAL ANALYSIS

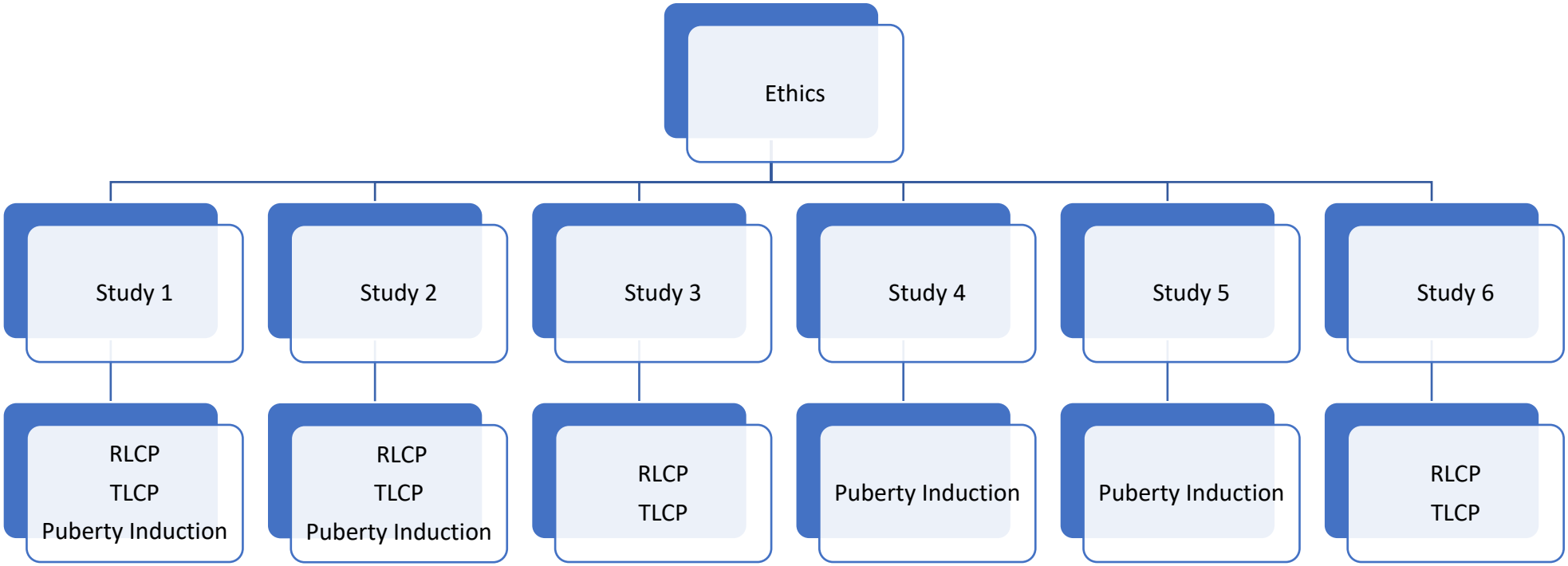
LIFE COURSE PROJECTS AND ETHICAL APPROVAL

Before I started this research, two overarching ethical approvals for work in this area were established in the department. The Turner Life Course Project (TLCP) and the Reproductive Life Course Project (RLCP) were established in 2014 and 2015 respectively. Their aim was to document the medical and health outcomes for adult women with TS and other endocrine pathologies such as POI. A questionnaire was completed covering health aspects, fertility experience, lifestyle and psychosocial outcomes. The ethics applications for the TLCP and RLCP had been approved by Chelsea Research Ethics Committee (TLCP reference: LO/2174; RLCP reference: 16/LO/0682). Studies 1, 2, 3 and 6 were conducted under the TLCP and RLCP ethical approval (Figure 2.1).

PUBERTY INDUCTION AND ETHICAL APPROVAL

Intervention with a fixed treatment protocol for induction of puberty was not adequately covered by the TLCP and RLCP. Therefore, a new ethical application was required for this aspect of the research. The ethical applications for this study were developed and managed using the Integrated Research Application System (IRAS). UCLH sponsorship of the project were sought and approved. The project was approved by the South West - Central Bristol Research Ethics Committee (Pubertal Induction Reference: 17/SW/0047) and the Health Research Authority (HRA). The HRA letter of approval can be seen in Appendix 4. Studies 1, 2, 4 and 5 were conducted under the Pubertal Induction ethical approval (Figure 2.1).

Figure 2.1. Figure demonstrating the ethical approvals under which each study was conducted. The Turner Life Course Project (TLCP) and the Reproductive Life Course Project (RLCP).



PARTICIPANT RECRUITMENT AND DIAGNOSTIC CRITERIA

Participants were recruited from the Paediatric Gynaecology, Reproductive Endocrinology and Reproductive Medicine clinics at University College London Hospital (UCLH).

Participants were recruited to the different studies with the following clinical presentations (Figure 2.2.).

Group A - Females presenting with primary amenorrhoea and puberty delay who had not received any previous oestrogen treatment. These participants underwent standardised oestradiol treatment as per Study 4.

Group B - Females presenting with either primary or secondary amenorrhoea and pubertal delay who were at various stages of exogenous oestrogen treatment (not standardised treatment).

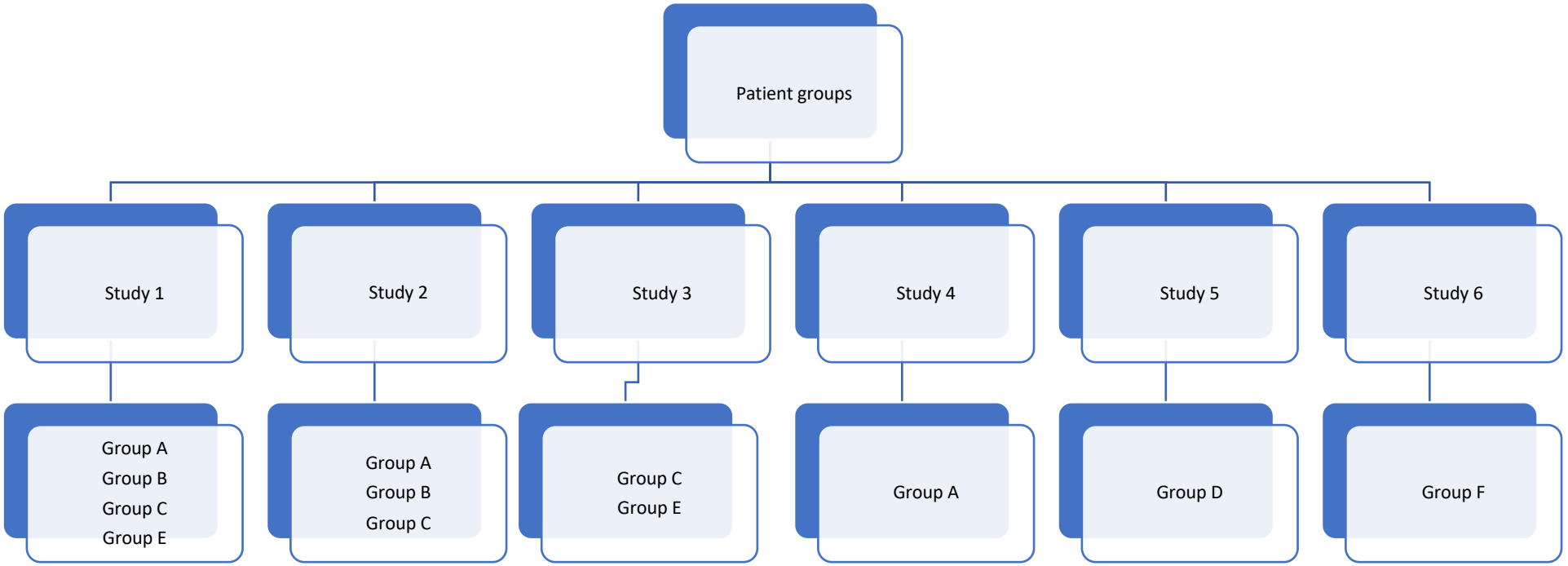
Group C - Females who had a history of primary amenorrhoea and pubertal delay who had completed pubertal induction treatment and were established on adult hormone replacement therapy.

Group D - Trans females who were receiving exogenous oestradiol treatment.

Group E - Females presenting with primary male factor subfertility with no pelvic pathology identified on ultrasound scan.

Group F - Females with Turner syndrome or Premature Ovarian Insufficiency who had undergone oocyte donation fertility treatment or who had a spontaneous pregnancy.

Figure 2.2. Figure demonstrating the diagnostic cohorts recruited for each study.



Patient were recruited for the studies with varying diagnosis. For the purposes of the studies:

- Premature ovarian insufficiency (POI) was defined as based on European Society of Human Reproduction and Embryology (ESHRE) guidelines, with diagnosis requiring 4 months of amenorrhoea/oligomenorrhoea in combination with a Follicle Stimulating Hormone (FSH) level of >25 IU/L on 2 occasions at least 4 weeks apart and hypoestrogenaemia (<44 pmol/L as measured by routine assay). The aetiology included idiopathic POI or those who had evidence of autoimmunity. For my study the karyotype was limited to those with 46, XX and excluding those if premature ovarian insufficiency was iatrogenic as a result of oncology or surgical treatment. Single gene mutations and autoimmune screening are included in routine clinic work up but were not included in this dataset and this subgroup was not the primary focus of research.
- Turner syndrome (TS) was diagnosed by karyotype analysis.
- Gonadotrophin Deficiency (GD) included hypothalamic amenorrhoea (HA), hypogonadotrophic hypogonadism (HH), and hypopituitarism (HP) with low gonadotrophins (<4 IU/L) and hypoestrogenaemia (<44 pmol/L) as measured by routine assay.
- Hypothalamic amenorrhoea was defined by a clinical history suggestive of a hypothalamic cause such as low body weight, in the presence of low/normal LH (4 IU/L) with low oestradiol concentration (<100pmol/L).
- Hypogonadotrophic hypogonadism was defined as low gonadotrophins (<4 IU/L) and hypoestrogenaemia (<44 pmol/L) in the absence of an obvious hypothalamic cause. As the clinical distinction between HH and constitutional delay of growth and puberty (CDGP) can be a challenge I assumed the

diagnosis of HH based on older age of presentation, other features of HH including anosmia, lack of hypothalamic precipitants and persistence of low gonadotrophins throughout the study period without evidence of recovery. For one patient a genetic panel result supported the diagnosis of HH, however genetic screening and serum Inhibin B levels are not routinely performed in our clinical service. The diagnosis and decision to start oestradiol treatment was following a multidisciplinary team review.

- Hypopituitarism was diagnosed with low gonadotrophins (<4 IU/L), hypoestrogenaemia (<44 pmol/L) and other clinical or biochemical evidence of pituitary dysfunction (affecting 2 or more pituitary hormones).
- Pubertal delay was defined as primary amenorrhoea with lack of thelarche with breast Tanner staging ≤2 (elevation of breast and papilla as small mound, areola diameter enlarged) by the age of 13 or primary amenorrhoea in the presence of secondary sexual characteristics by the age of 15 years.
- 17 beta hydroxylase deficiency was diagnosed with biochemical and genetic analysis.

OESTRADIOL ASSAYS

For the purposes of my research I used two different oestradiol assays:

1. Standard oestradiol concentrations processed at the biochemistry laboratory at University College London Hospital as per routine protocol (Roche Electrochemiluminescence immunoassays; Elecsys LH and FSH and Elecsys Estradiol III). The standard oestradiol assay had a lower detection limit of 44pmol/L.
2. Sensitive oestradiol concentration processed at South Manchester Hospital biochemistry laboratory using a sensitive LC-MS/MS (Liquid chromatography

tandem mass spectrometry) assay. The sensitive oestradiol assay had a lower detection limit of 10 pmol/L. This assay was only used for study 4.

STUDY 1 - EXAMINATION OF THE REPRODUCIBILITY OF UTERINE DIMENSIONS USING TRANSABDOMINAL ULTRASONOGRAPHY COMPARED TO TRANSVAGINAL ULTRASONOGRAPHY AND MRI

Participants

In this prospective observational study, participants were recruited as part of Study 3 and 4 or for the purposes of examining ultrasound reproducibility. Participants included those undergoing or who had previously undergone pubertal induction and nulliparous women with male factor subfertility having routine ultrasonography as part of their fertility investigations.

Ultrasound Examination protocol

An ultrasound protocol was designed and a standardised protocol for measurements of the uterus developed.

Ultrasonography was performed using a Voluson E8 ultrasound machine. For a transabdominal approach a full bladder technique was employed and a C4-8-D 2-7MHz wide band convex transducer was used. For a transvaginal approach a 3D RIC5-9-D transducer was used with an empty bladder. The standardised measurements are shown in Figure 2.3 and 2.4 and recorded in mm.

In the longitudinal plane the vagina (between the bladder anteriorly and the rectum inferiorly), the cervix, the uterine body and the endometrium (seen as a

continuum, from the cervical canal to the fundus) were identified and the position of the uterus (anteverted or retroverted) noted.

In the longitudinal plane the uterine body length (Figure 2.3 line A - from the serosal edge of the myometrium to the level of the internal os - level of the insertion of the uterine arteries) and cervical length (Figure 2.3 line B - internal os to the external os) were added together to provide a total uterine length measurement. In the sagittal section, the anterior posterior (AP) measurement of the uterine body and cervix (Figure 2.3 lines C & D- mid sagittal section from the anterior to posterior serosal edge at the largest dimension of the uterine body and cervix, perpendicular to the length) were recorded. In the transverse plane the width of the uterine body (Figure 2.4 line A -level of the interstitial portions of the fallopian tubes/broad ligament from serosa to serosa) was measured. The ellipsoid formula was used to calculate uterine volume (Volume (ml) = Transverse x AP x Length x 0.5233). The fundal cervical AP ratio was calculated, and a mature uterine configuration was classified when the AP of the uterine body was larger than that of the cervix, with a ratio > 1. The endometrial thickness was measured between the echogenic interfaces at the endometrium - myometrium junction. The maximum anterior - posterior distance was measured.

Each set of uterine measurements was taken 3 times following a standard order of measurements. A complete set of measurements was taken prior to starting on the subsequent 2 sets of measurements. The mean of the 3 measurements was used for documentation and statistical analysis.

The ultrasound data was recorded on a clinical/ultrasound database (Viewpoint ultrasound database, version 5.6.8.428, Bilderararbeitung, GmbH).

Figure 2.3. A longitudinal transabdominal ultrasound image of the uterus showing landmark points for measurement

Line A - uterine body length, line B - cervical length, line C - anterior posterior measurement of the uterine body and line D - the anterior posterior measurement of the cervix.

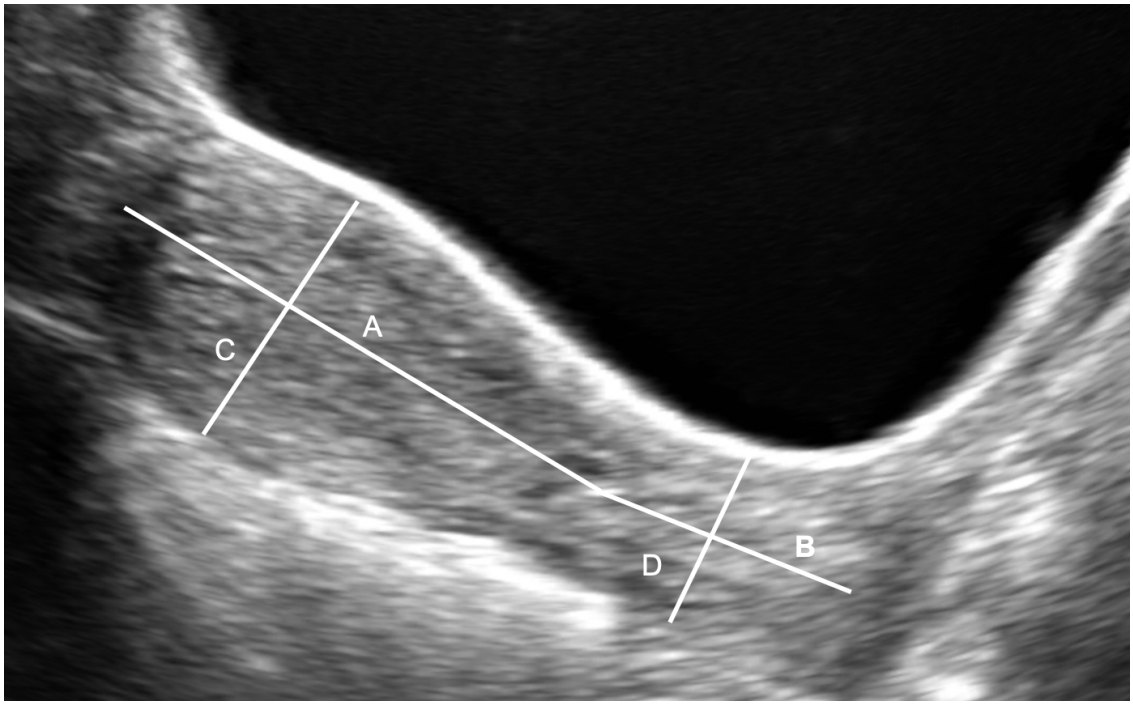
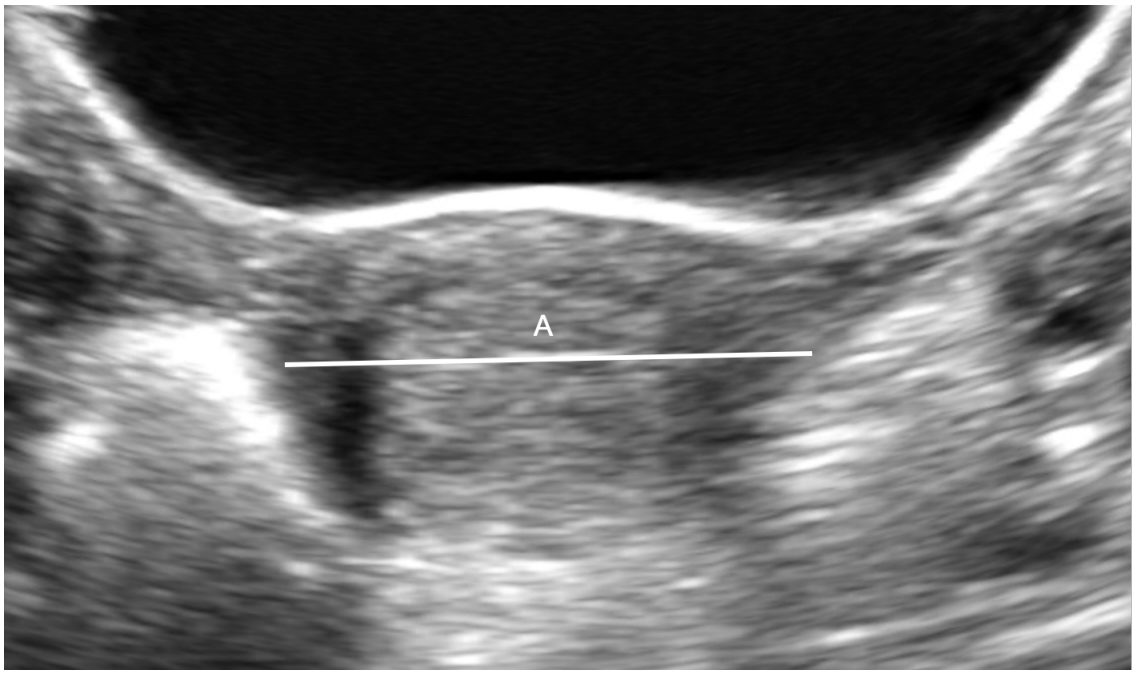


Figure 2.4. A transverse transabdominal ultrasound image of the uterus showing landmark points for measurement

Line A - width of the uterine body.



Magnetic Resonance Imaging (MRI)

MRI examinations were performed using 1.5T scanner with a phased array body coil. Prior to the examination all participants were given 20-40mg intravenous (IV) buscopan to suppress bowel movement. All examinations contained a T2 sagittal and axial section of the pelvis and T2 spin echo. Uterine examinations were completed by a single Consultant Radiologist who is a subspecialist in adolescent imaging (observer C) using a standardised measurement protocol. The ellipsoid formula was used to calculate uterine volume measurements.

Inter and Intra observer reproducibility of transabdominal ultrasound

Transabdominal ultrasound was completed by observer A (EB) and B (DM), both trained in transabdominal gynaecological ultrasound. The TAUS examinations were completed initially by observer A and then observer B on the same clinic visit, so that bladder filling and ultrasound machine settings were constant. Following the completion of the examination by observer A, observer B entered the room and was blinded to the clinical history and measurements taken by observer A.

To assess intraobserver reproducibility TAUS examinations were completed twice, with a delay of approximately 30 minutes, by observer A. A longer delay was not deemed possible due to the possible change in bladder filling.

Agreement between modalities

To assess the agreement between TAUS and TVUS, observer A performed a TAUS first followed by a TVUS scan, after complete bladder emptying, on the same individual during the same clinic visit. To compare TAUS and MRI, participants recruited for the formal pubertal induction study had a pelvic MRI

scan and TAUS after 8 months of treatment. The TAUS was completed by Observer A and the MRI was reported by Observer C.

STUDY 2 - CHALLENGES IN DEVELOPING A QUANTITATIVE METHOD OF MEASURING BREAST DEVELOPMENT USING 3D IMAGING - AN EXAMPLE OF A NOVEL METHOD FOR USE IN INDUCED BREAST DEVELOPMENT WITH EXOGENOUS OESTROGEN

3D breast imaging protocol development

3D breast images were taken using a 3dMD Photogrammetric System (3dMD Inc., Atlanta, GA, USA). Calibration of the camera took place prior to each imaging session as per the manufacturer's guidelines. As this was a novel technique several iterations were needed to ensure optimal image quality and a standardised protocol was developed.

It was visually apparent that different sitting positions, with alteration in head and arm position produced artefact in anterior chest wall and breast tissue movement. Therefore, I designed and created a bespoke postural support which was placed behind the participant's back, keeping the participant upright and arm position static above their head as seen in Figure 2.5 and 2.6. The head was kept in a neutral position and images were taken in full inspiration. A separate image was taken of each breast ensuring both frontal and lateral view. Images should include a view from the sternal notch to the inferior rib and the frontal view should include a free lateral border.

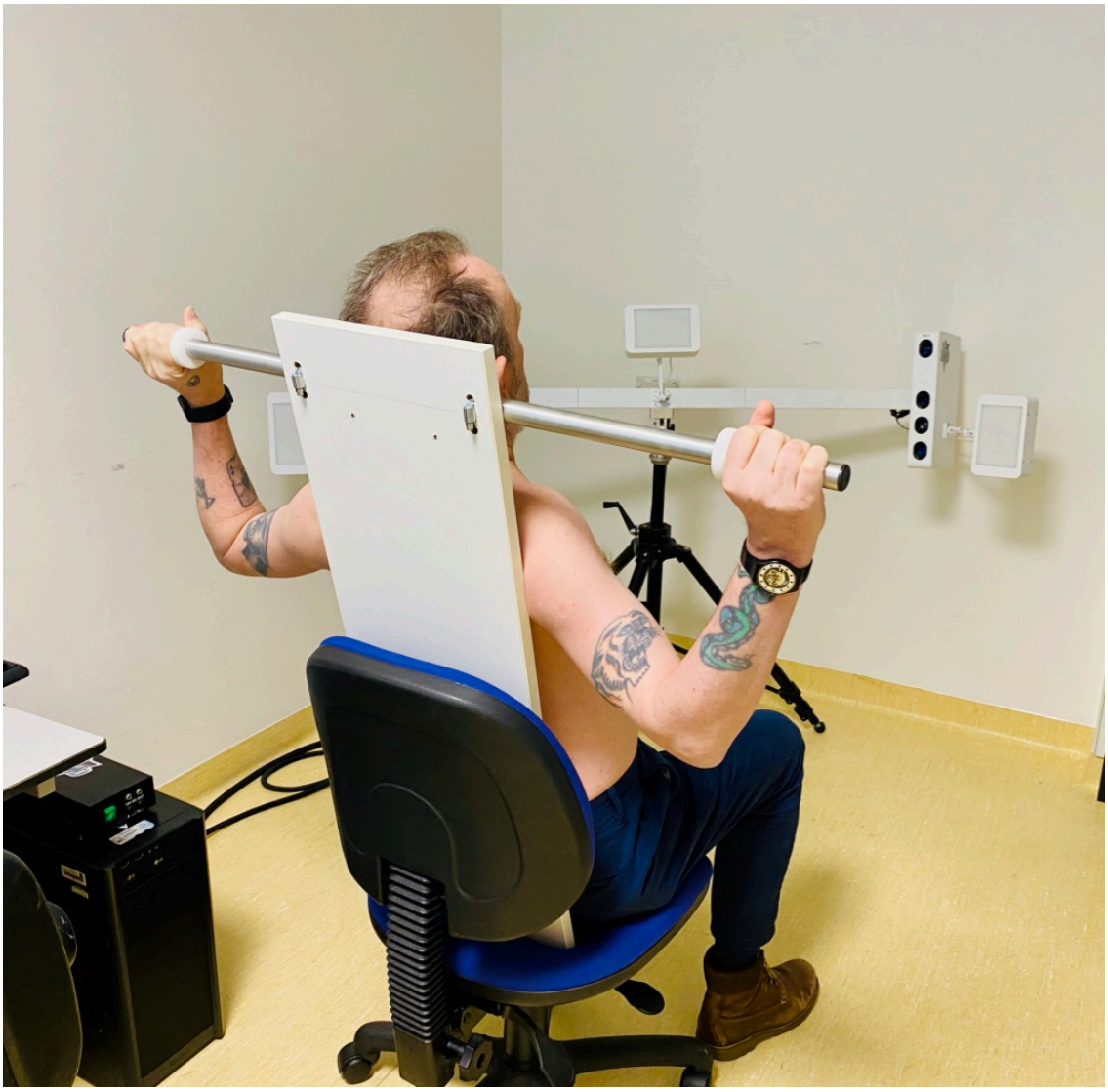
Differential breast volume between two breast images was calculated using Robin software (Cloud RR3D. Voxel processing package (http://www.robins3d.co.uk). Images were superimposed and aligned manually

by a single observer (EB). Alignment was ensured using a 'Differences of Surfaces' technique which provides a quantitative measurement between the two surfaces and displayed as a colour overlay on the 3D breast image. Using an upper limit of 0.5 mm for the differences in surfaces, the border of the static chest wall around the breast was delineated and marked on to the image to provide the projected area of breast tissue. Within the projected area of breast tissue, the mean difference of the surfaces was calculated. The breast volume (mls) was then calculated by multiplying the mean distance between the surfaces by the projected area.

Figure 2.5. Image demonstrating the bespoke board designed and manufactured to keep the participant upright and arm position static above their head

Figure 2.6. Image demonstrating the positioning of participants using the bespoke postural support in front of the 3dMD Photogrammetric System

Participants were seated in an upright position against a bespoke postural support, with their arms in a static position above the head. The head was kept in a neutral position and images were taken in full inspiration.



Static breast imaging

To ensure that breast volume change was not secondary to movement artefact due to participant positioning or as consequence of poor image alignment, a male subject underwent image acquisition on different days and a total of 9 different images were taken (A-I). Seventeen random different combinations of images were used and superimposed and the mean difference of surfaces in the area of interest calculated. No change in breast volume was expected. Figure 2.7

demonstrates two images taken of the male subject taken on two of the separate occasions. Figure 2.8 represents the same two images superimposed with no change noted within the breast area (represented as a colour overlay).

Figure 2.7. Two images of the same male participant taken on different occasions where no volume change in the breast region would be expected

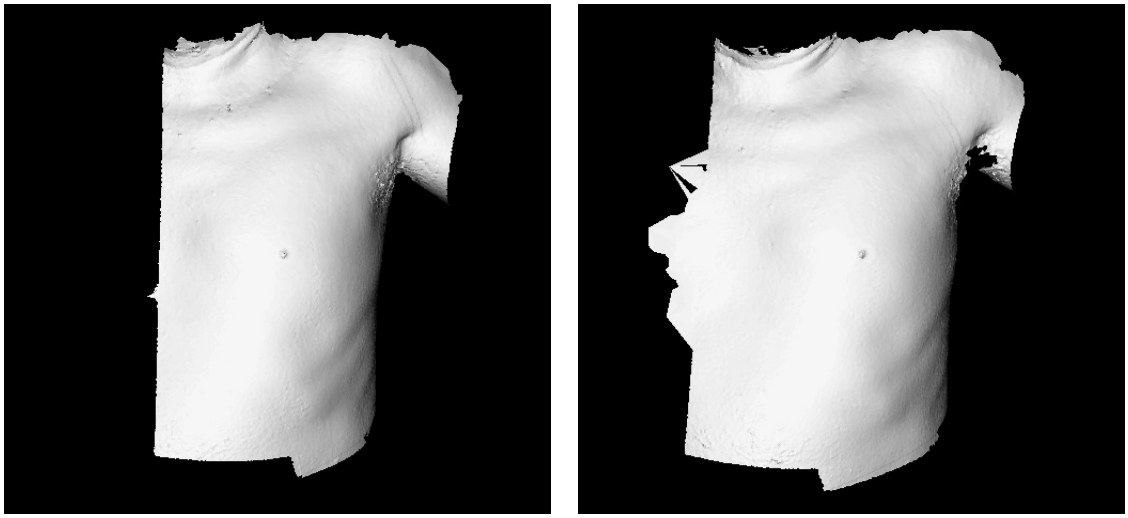
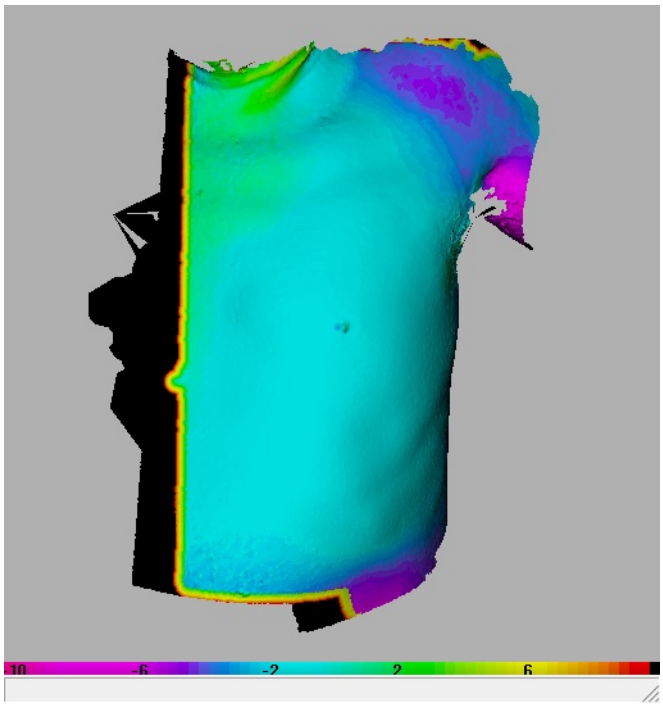


Figure 2.8. The differences of surfaces image represented

The light blue colour demonstrating the minimal area of change and the darker colours representing increasing change.



Intraobserver variability of 3D breast volume measurements

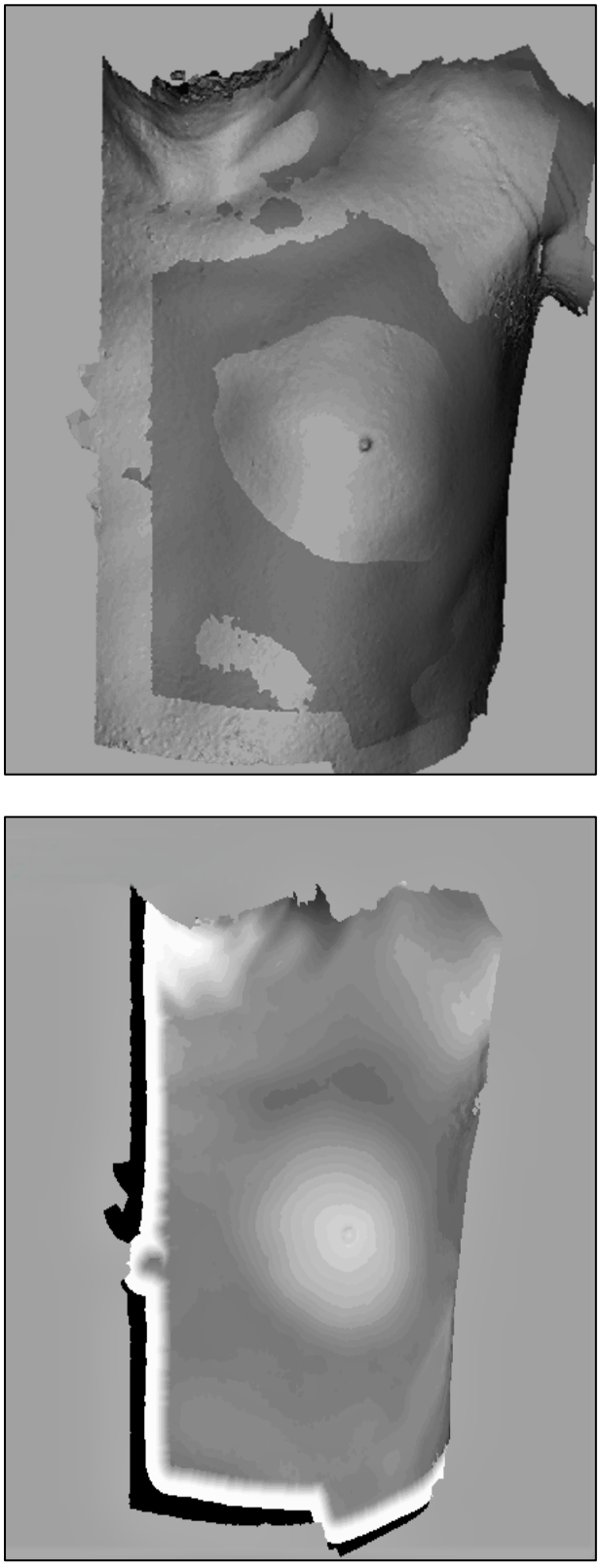
To assess the intraobserver variability of 3D breast volume measurements the process of image manipulation, alignment and acquisition of measurements were completed by the same observer (EB) twice with a period of 2 weeks at least between measurements.

Comparison to computer-generated reference volume

To simulate a change in breast volume over time and produce an artificial reference volume, two randomly chosen images of the male participant (image A and D) underwent thin plate spline warping to produce a displacement in the breast area, with maximal displacement at the nipple. A computer generated 5 and 10 mm displacement of the breast area was created for both image A and image D. The difference in breast volume between image A and displaced image A was then calculated. This was repeated with Image D. Aligning the displaced image directly to the unwarped counterpart served to remove error due to patient position and operator image alignment. This produced a 'reference volume' to use for method validation in the same vein as using excised tissue water displacement (212-214), and also implant volume (215) in the adult population.

Operator manual image alignment was subsequently completed by myself blinded to the reference volume produced by computer generation. The warped image A and D (5 and 10mm) was superimposed manually in turn with 8 unwarped images (B-I) of the same individual taken on different occasions and the differential volume calculated. Figure 2.9 and demonstrate the two images superimposed followed by the differences of surfaces image (represented in grey scale).

Figure 2.9. A static image of male participant (dark grey) superimposed with the 10mm warped image (light grey). The differences of surfaces image represented as grey scaling The grey scaling would usually have a colour overlay, but in this black and white image the lighter white area demonstrating the maximum area of volume change.



Application in participants undergoing induced breast development

A total of 29 participants undergoing induced breast development with exogenous oestradiol were recruited. Twenty-six presented with primary amenorrhoea and pubertal delay (8 with TS, 8 with POI, 7 with HH, 2 with HA and 1 with 17 beta hydroxylase deficiency) and 3 had secondary amenorrhoea and incomplete breast development (2 with TS and 1 with POI). Thirteen participants were part of study 4, and 16 were routine clinic patients opportunistically recruited for the assessment of reproducibility. Breast imaging was completed twice at 2 time points during oestradiol treatment with timing dependent on clinical attendance. There was no standardised protocol for oestradiol treatment as participants were at various stages of the treatment and treatment was governed by routine clinical practice.

Both left and right breast images were reviewed and a total of 58 images were used for reproducibility analysis. The process of image alignment and breast volume calculation was repeated twice by the same observer. The differential breast volume was calculated as above.

Development of landmarking reproducibility

In the early phases of technique development, the possibility of landmarking in addition to the effects of participant posture and sitting position on breast tissues were examined in detail. The motivation for landmarking was to be consistent and in line with the methodology documented in other papers to generate breast volume, rather than developing a novel technique. Furthermore, the landmarking points will be used to construct a methodology and software to allow shape analysis on the breast.

To explore the feasibility and reproducibility of landmarking, images were taken of a male subject. No breast change nor weight change would have contributed

to breast volume, therefore providing a surrogate model of minimal breast development, as it would be predicted that landmarking with minimal breast tissue and indistinct tissue borders would be more challenging.

Multiple attempts at landmarking were undertaken using various methods on the male participant including both placements directly on the individual and digital marking on the image. An amalgamation of the most common breast tissue landmarks used in the literature are shown in Table 2.1 (190, 200, 202, 212, 213, 222). Multiple combinations of landmarks were trialled, systematically excluding those which were impossible to reproduce reliably. Initially on the male participant, small sticker dot placement and marking were completed directly on the chest wall, as seen in Figure 2.10. The landmarking was not reliable upon visual inspection and it was felt that these techniques would not have good patient acceptance due to the act of marking the chest wall and the time of exposure. This is turn would compromise reproducibility further due to time pressure. Ultimately marking on the digital image was decided upon. The 7 landmarks that were used for my protocol are shown in Figure 2.11.

Table 2.1. The standard landmarks used in the literature defining the breast border

Position of landmark	Anatomical definition
Sternal Notch	The visible dip at the base of the neck, between the clavicles
Medial Breast	A point in line with the nipple, approximately 1 cm from the medial extent of the breast
Medial infra Mammary Fold (IMF)	A point approximately 1-2 cm from the medial extent of the IMF
Bottom of IMF	A point approximately 1-2 cm below the bottom of the IMF
Lateral IMF	A point approximately 1-2 cm from the lateral extent of the IMF
Lateral Breast	A point in-line with the nipple, at the lateral extent of the breast
Anterior Axillary Fold	At the lateral off shoot of the pectoralis muscle
Clavicle	A point near the lateral extent of the clavicle, at the same height as point 1 (1 cm below clavicle)
Nipple	Nipple protrusion
Mid axillary line	Mid axillary at the level of the nipple

Figure 2.10. Frontal and lateral image of the chest wall of the male participant demonstrating direct landmarking on the chest wall of the breast boundaries

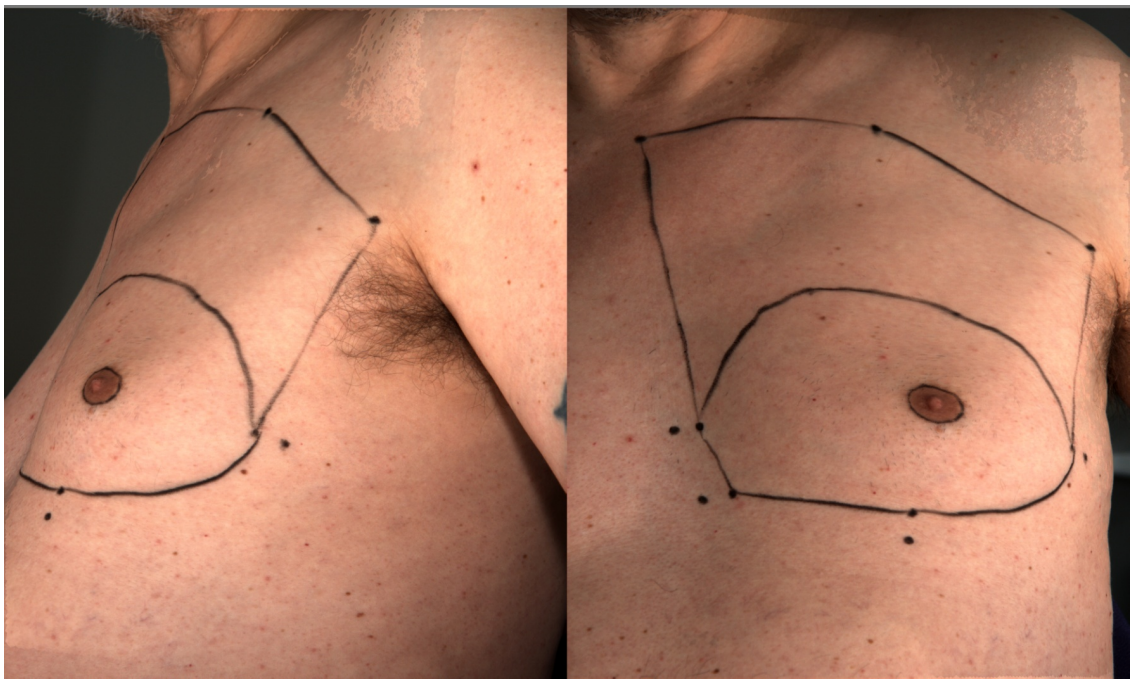
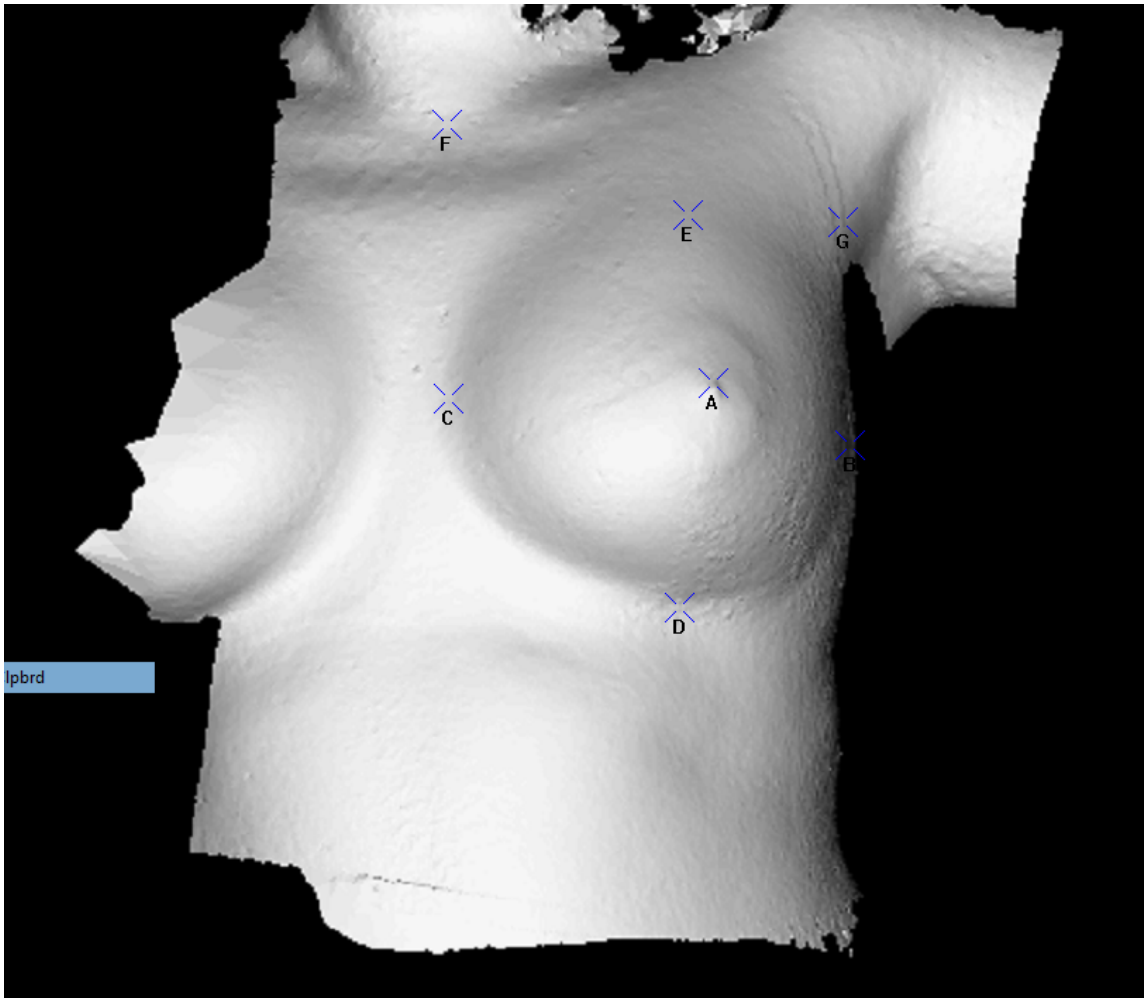


Figure 2.11. Positions of landmarks placed directly on the digital image

A = Nipple (Nipple protrusion), B = Lateral Breast (A point in line with the nipple, at the lateral extent of the breast), C = Medial Breast (A point in line with the nipple, at the medial extent of the breast), D = Infra Mammary Fold (A point at the bottom of the Infra Mammary Fold, E = Superior Breast (A point at the superior aspect of the breast), F = Sternal notch (The visible dip at the base of the neck, between the clavicles), G = Anterior axillary line (The lateral off shoot of the pectoralis muscle).



Breast images were also completed for 14 females recruited who had completed pubertal induction and therefore landmarking was also trialled on those with more mature breast development. Using the digital frontal image 3 different technique of landmarking were examined sequentially. Firstly, the static image was placed in a vertical position with as little rotation as possible and the points were placed. Secondly, to facilitate better visualisation of the breast borders a dynamic approach was trialled whereby the image was moved and rotated and then

marked. Thereafter I recognised that rotational error affected landmarking and therefore thirdly I tried delineating the border with a colour ring first prior to landmarking. The markers were placed on each image twice by the same observer (EB) with a 2-week interval. The difference in placement markers was calculated in mm.

STUDY 3 - UTERINE VOLUME AFTER INDUCTION OF PUBERTY IN WOMEN WITH HYPOGONADISM

Participants

In this retrospective cross-sectional study, the inclusion criteria for participant recruitment was being over the age of 16 with a history of primary amenorrhoea due to hypogonadism, who had previously received exogenous oestrogen for induction of puberty, were nulliparous and were established on Hormone Replacement Therapy (HRT).

Women with hypogonadism were considered in three diagnostic groups. Turner syndrome (TS), Premature Ovarian Insufficiency (POI) and Gonadotrophin Deficiency (GD) including hypothalamic amenorrhoea, hypogonadotrophic hypogonadism, and hypopituitarism.

The reference group consisted of nulliparous women with spontaneous puberty who presented to the Reproductive Medicine clinics with male factor subfertility and had regular menstrual cycles, no relevant gynaecological history nor pathology identified on pelvic ultrasound.

Pelvic Ultrasound

Ultrasonography was performed as described above by a single observer (EB) using a Voluson E8 ultrasound machine. Either a transvaginal approach or transabdominal approach was used depending on whether the women had been sexually active and by patient choice. Scans were performed randomly in the menstrual cycle and the timing of the scan was recorded as being in the oestrogen only phase or oestrogen plus progesterone phase of the hormone cycle.

In the absence of a universally accepted definition of adult uterine size I chose to use the 5^{th} centile of ultrasound measurements from the reference group.

Examination and clinical data

Examination included height, weight and BMI (kg/m^2) measurements. Clinical data was recorded using both review of notes and patient recall and comprised a full medical history, age of starting oestrogen therapy, use of growth hormone (GH), prior evidence of breast development, type and dose of oestrogen started, titration details, age of menarche, type of bleed (induced or spontaneous), final dose of oestradiol, duration of unopposed oestrogen therapy and current hormone replacement. Serum oestradiol levels were recorded from 22 women taking HRT preparations containing 17β oestradiol.

STUDY 4 - VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM.

Participants

In this prospective observational study, I chose to investigate females with primary amenorrhoea and pubertal delay due to various different aetiologies, rather than restrict to a single diagnosis, to reflect the clinic population. Eligibility criteria were females presenting with primary amenorrhoea and pubertal delay (Tanner breast stage < 2), or in cases of TS evidence of hypogonadism. Participants were recruited with premature ovarian insufficiency (POI), Turner syndrome (TS), 17 beta hydroxylase deficiency and hypogonadotrophic hypogonadism (HH). Thyroid and prolactin levels were normal for all. Exclusion criteria included previous oestrogen treatment and females with Polycystic Ovarian Syndrome (PCOS), or hypogonadism secondary to oncology treatment or chronic medical illness.

Examination and Investigations

At baseline, a full medical history was obtained including details of diagnosis, co-morbidities, medications and family history.

Participants were assessed every 2 months for a total of 8 months and then at 12 months. They were seen by the same clinician (EB) on each occasion. At each visit the clinical history was updated, anthropometric measurements were taken for height (cm), weight (kg), BMI (kg/m^2), hip and waist measurements (cm). Height Z scores and height velocity (cm/year) were calculated. Body fat

measurements (%) were measured by Bioelectrical Impedance Analysis (Tanita machine type BC-418 MA serial number 11030191).

Height was measured using a stadiometer to the nearest mm and taken with the subject's feet together and the head in a horizontal plane. The heels, buttocks and shoulder blades should be against the stadiometer. Weight was measured to the nearest 0.1kg using electronic scales. Body Mass Index (BMI) was calculated using the formula kg/m^2 and blood pressure was measured in mmHg, using a suitable sized cuff. Waist circumference was measured in mm, between the lowest border of the rib cage and the upper border of the iliac crest. The measurement was taken with the participant standing and at the end of a normal expiration. Hip circumference was measured in mm at the level of anterior superior iliac spine.

Bone age monitoring was performed in routine paediatric clinic visits when appropriate but was not analysed in this work.

Pubertal assessment was completed using Tanner staging for breast. Blood samples were drawn for Follicle Stimulating Hormone IU/L (FSH), Luteinizing Hormone IU/L (LH) and oestradiol. Bloods tests at initial presentation and at 8 months were taken for Insulin Like Growth Factor -1 nmol/L (IGF-1), Thyroid Stimulating Hormone mIU/L (TSH) and Free T4 pmol/L (FT4). Samples were timed around clinic appointments. Samples were processed at the biochemistry laboratory at University College London Hospital as per routine protocol (Roche Electrochemiluminescence immunoassays; Elecsys LH and FSH and Elecsys Estradiol III). The standard oestradiol assay had a lower detection limit of 44pmol/L.

Serum samples were stored at each visit for E2 using a sensitive LC-MS/MS (Liquid chromatography tandem mass spectrometry) assay at South Manchester Hospital biochemistry laboratory. The sensitive oestradiol assay had a lower detection limit of 10 pmol/L (126). The oestradiol levels derived from the LC-MS/MS were used in analysis. Whilst oestrone is not anticipated to be present in significant quantities with the use of the transdermal oestradiol patch, the sensitive LC-MS/MS assay used in this study showed no significant cross reaction between oestrone and oestradiol (126).

Pelvic Ultrasound

Ultrasonography was performed as described above. Uterine parameters at baseline and after 8 months of oestradiol treatment were compared to published normative data adapted from Hagen et al. which assessed uterine size compared to Tanner staging in females undergoing spontaneous puberty (46). Uterine parameters were also compared to 5^{th} percentile reference uterine size data from those who had undergone spontaneous puberty in study 3.

3D Breast Volume

3D breast images were taken as described above using 3dMD Photogrammetric System. The mean of 2 measurements taken for each breast was calculated. The final breast volume change was the sum of the change in left and right breasts. Using this technique, the first image at baseline was assigned breast volume of zero.

To assess the intraobserver variability of 3D breast volume measurements, the process of image manipulation, alignment and acquisition of measurements was

completed by the same observer twice with a period of 2 weeks between measurements. A total of 30 separate breast images were reviewed.

Dual emission x-ray absorptiometry

A dual emission x-ray absorptiometry (DEXA) of total hip and L1-L4 spine was performed prior to the initiation of therapy and after 8 months of treatment. The Z score, bone mineral concentration (g) (BMC), and bone mineral density (g/cm^2) (BMD) were recorded. The DEXA was performed using a Hologic Discovery A (S/N 83799) machine with current software version 13.6.0.2.

Intervention

A standardised treatment protocol was followed in all subjects for 8 months. Transdermal 17β Oestradiol (17βE) was used (Evorel™). Evorel 25 has a surface area of 8 cm^2 and contains 1.6mg E2, corresponding to an estimated absorption of 25mcg per 24 hours. Each 25mcg patch was cut in half to achieve a starting dose of 12.5 mcg/24h transdermal 17βE (changed twice weekly) which was given for 4 months. After 4 months, the dose was increased to 25 mcg/24h (changed twice weekly) and continued until 8 months.

The starting dose and escalation protocol were selected to reflect the relatively late age of presentation in the clinic's experience. Most protocols and guidance for pubertal induction advocate low starting oestradiol doses, with body surface area (BSA) dose calculations, as this is based on treatment initiation occurring at a similar physiological age as spontaneous puberty (117, 119). In my cohort however 14/16 participants were 14 years or older when diagnosis and treatment initiation occurred and therefore, consistent with current agreement, a higher starting dose and more rapid escalation was chosen (115, 122).

Between 8 and 12 months the dose was either increased to 37.5mcg patch or 50 mcg patch depending on clinician assessment, departing from fixed protocol.

Investigations were not timed according to patch change time as it was assumed that any short-term fluctuations in oestradiol around patch changes would not affect progressive uterine and breast growth (137, 138). Compliance was assessed by verbal assessment at each visit by a single observer. There were no reported side effects limiting the patch use.

STUDY 5 - QUANTITATIVE ASSESSMENT OF BREAST VOLUME USING 3D IMAGING IN TRANS FEMALES: A PILOT STUDY

Participants

In this prospective observational study, trans females who were on gonadotrophin releasing hormone agonists (GnRHa) and about to start on gender-affirming oestrogen therapy were recruited.

Examination and Investigations

Participants were assessed prior to the start of oestrogen treatment and after 6 months by the same clinician (EB). Anthropometric measurements were taken for height (cm), weight (kg) and BMI (kg/m^2). Blood samples were drawn for oestradiol (pmol/L). Samples were processed at the biochemistry laboratory at University College London Hospital as per routine protocol (Roche Electrochemiluminescence immunoassays; Elecsys LH and FSH and Elecsys Estradiol III).

3D breast images were taken as described above and breast volume was calculated using the 'Differences of Surfaces' technique. This technique did not

require landmarking. Breast images were taken prior to the initiation of oestrogen treatment and at 6 months post treatment initiation. The final differential breast volume change was the sum of the change in left and right breasts. Using this technique, the first image at baseline was assigned breast volume of zero.

STUDY 6 - FERTILITY AND PREGNANCY OUTCOMES IN WOMEN WITH TURNER SYNDROME AND PREMATURE OVARAIN INSUFFICIENCY

Participants

In this retrospective single centre cross-sectional study, participants were recruited from the dedicated Turner syndrome clinics of the Reproductive Medicine Unit at University College London Hospital (UCLH). These clinics have attendance from throughout the UK however fertility treatment and antenatal services usually took place at a hospital local to the participant's home.

The inclusion criteria for recruitment were age above 16 years, a diagnosis of Turner syndrome or Premature Ovarian Insufficiency (POI) with OD or spontaneous pregnancy. Turner syndrome was diagnosed clinically and confirmed with karyotype analysis.

Clinical data

Women who had tried to conceive or who had been pregnant were identified by clinic staff and followed up by structured interview (Appendix 5) and detailed note review.

The TS karyotype was recorded and for the purposes of analysis for outcomes was categorised into 45, X or other. Age at each cycle of oocyte donation or at each pregnancy was collected in addition to their current age. Height, weight and

BMI (kg/m^2) measurements were recorded prior to the first fertility treatment or first pregnancy. The presence of treated hypothyroidism prior to fertility treatment or pregnancy was recorded (excluding those who had subclinical hypothyroidism treated at the time of fertility treatment). Menstrual cycle history included a record of primary or secondary amenorrhoea or regular menstrual cycles. The presence of hypertension or diabetes prior to fertility or pregnancy was also recorded.

The Turner syndrome clinical care guidelines, published in 2024, provide guidance on pre pregnancy care and echocardiogram assessment based on aortic size index (ASI) and cardiac pathology (82, 120). This level of care was already in place at UCLH prior to formalization of the recommendations, having been developed over the preceding 20 years. Regular echocardiogram assessment for women with Turner syndrome every 2-5 years is standard routine health surveillance at UCLH with additional pre-pregnancy assessment. Furthermore, a detailed plan for recommended antenatal monitoring including cardiology surveillance is provided once pregnancy is confirmed, as most women will receive antenatal care in their local area.

Cardiac status in TS included the presence of cardiac pathology (bicuspid aortic valve or coarctation and repair of the aorta). Echocardiogram assessment, aortic size index (ASI) and cardiology assessment prior to fertility treatment or pregnancy was recorded.

Fertility treatment

Fertility treatment occurred between the years of 1986 -2023. Given the changes in practice over time, moving towards elective single embryo transfer (eSET) and the fact that multiple fertility centres with varying protocols contributed to this data, variables predicting outcome were restricted to the recipient characteristics. The

age of donor, endometrial preparation protocol and thickness, number and stage of embryos, the use of fresh or vitrified embryos and sperm source were not included in the analysis.

Primary outcomes analysed were pregnancy rate and live birth rate per cycle started, with the delivery of multiple pregnancy counted as one live birth event, which is consistent with the reporting of Human Fertilisation Embryo Authority (HFEA). Treatment cycles were categorised into first cycles and subsequent cycles.

Pregnancy data

Pregnancies were recorded between 1979 – 2023. Pregnancy history included mode of conception, either spontaneous conception or with OD. Miscarriage was defined as a pregnancy loss prior to 23 weeks and 6 days gestation not including termination of pregnancy or ectopic pregnancy. Live birth was defined as the delivery of a live baby (or babies) after 24+0 weeks' gestation. Intrauterine death (IUD) was defined by fetal demise after 24 weeks gestation. Termination of pregnancy and the reason, if known, was recorded.

Gravidity was the number of pregnancies and parity was defined as the number of pregnancies which developed past 24 weeks gestation. For outcomes, pregnancy was categorised into first pregnancy and subsequent pregnancies/deliveries. For those with POI, only pregnancies after the diagnosis were included for analysis, although prior pregnancies were recorded for the purpose of defining gravidity and parity.

Pregnancy complications were recorded for those that developed past 24 weeks and resulted in live birth or IUD. All pregnancies, including multiple pregnancies

were recorded, however given the known increased incidence of adverse maternal and neonatal outcomes associated with multiple pregnancy, only singleton pregnancies were included in the analysis of pregnancy complications (319, 320). Hypertensive disorders of pregnancy consisted of either pregnancy induced hypertension (HTN) and/or pre-eclampsia (PET) and excluded those who had pre-existing hypertension. The development of gestational diabetes mellitus (GDM) was recorded and excluded those with pre-existing diabetes mellitus. Mode of delivery was categorised into vaginal delivery including both spontaneous and instrumental delivery and LSCS, both elective and emergency.

Neonatal outcomes, for each singleton pregnancy resulting in live birth after 24 weeks gestation, included gestational age (weeks) at time of delivery with preterm birth being defined as delivery less than 37+0 weeks' gestation (321). Birthweight percentile was calculated (322) and small for gestational age was defined as a birthweight less than the 10^{th} centile. Information regarding the health of offspring was included and the presence of chromosomal anomalies was recorded.

Comparison to normative data

Outcome data for TS and POI spontaneous pregnancies were compared to normative general population data. The rate of miscarriage in the general population is 15.3% (323). Normative data for singleton spontaneous pregnancies was adapted from Storgaard et al. (230). The percentages used in this metanalysis of each original paper for spontaneous singleton pregnancies were used, and a mean calculated. The original papers consisted of national cohort studies of over 30,000 spontaneous conceived singleton pregnancies (324, 325).

Uterine size

Uterine size prior to pregnancy was recorded in 55 women (32 TS, 23 POI) using a standardised protocol for uterine measurements as above. A single observer (EB) either completed the transvaginal ultrasound (n=13) or reviewed stored images and applied a standardised measurement technique to assess uterine size (n=42).

STATISTICAL ANALYSES - GENERAL

Statistical analysis was completed using SPSS version 27 for Mac. The Shapiro-Wilk test was used to test normality of continuous variables. For variables that were normally distributed mean and 95% confidence intervals or standard deviation were described and a paired T test or independent T test were used to compared differences in means. If variables were not normally distributed variables were described in frequencies and percentages or median, 5^{th} and 95^{th} percentile and non-parametric tests were used for analysis. Chi squared analysis was used to examine the differences in categorical variables. Relationships between continuous variables were analysed using Spearman Rank and Pearson's correlation.

Intraobserver reproducibility, interobserver reproducibility and agreement between modalities were assessed using both the Bland Altman limits of agreement and the intraclass correlations (ICC) methods. The Bland Altman method allows the mean difference to be plotted against the mean of the two measurements and 95% limits of agreement (mean difference +/- SDx1.96) to be analysed (326). ICC estimates and the 95% CI were calculated using a two-way mixed effects model with absolute agreement model. Based on the 95% CI of the ICC estimates values < 0.5 indicate poor reliability, 0.5 - 0.75 indicate moderate

reliability, 0.75 - 0.9 indicate good reliability, and > 0.90 indicate excellent reliability (327). Statistical significance was defined as P value < 0.05.

STUDY SPECIFIC STATISTICAL ANALYSIS

Study 2

Accuracy, comparing the known breast volume to the differential volume calculated by manual alignment was assessed using the one-way T test. The one-way T test was also used to examine the differences in landmark placement for the 3 different techniques trialled.

Study 3

Age, BMI, weight, uterine volume measurements as well as oestrogen initiation age and menarche age were not normally distributed.

A power calculation was performed based on the data from Tsilchorozidou et al. (50). A difference of 12mm of uterine length between the diagnostic group and the control group was deemed clinically significant. Using a p value of 0.5 and power of 0.9 a minimum sample of 15 was calculated, which was exceeded to allow for possible breeches from protocol or loss to follow up.

Study 4

All primary outcome measures were normally distributed, with the exception of age of treatment initiation and breast differential volume.

To determine which uterine parameter correlated most closely with serum oestradiol levels at 8 months, a step wise regression model was used with pair-wise exclusion for missing data. The model included uterine length, AP uterine

body, transverse, uterine volume and endometrial thickness measurements at 8 months. Thereafter, to assess which factor best predicted uterine and breast development at 8 months step wise regression models were used with pair-wise exclusion for missing data. The variables included were age of treatment initiation and the following data points at 8 months : uterine transverse diameter, oestradiol concentration, differential breast volume, height Z score and BMI. Results were presented as beta standardised coefficients.

Study 6

Height, weight, BMI, age at time of 1st fertility treatment, number of fertility cycles, age at pregnancy, gravidity, parity, gestational age and birth weight percentiles, uterus anterior posterior measurement and uterine volume were not normally distributed. Age at time of OD cycle, birth weight, uterine length and transverse uterine measurements were normally distributed.

For binary fertility and pregnancy outcomes, regression analysis with forward selection (likelihood ratio) was used. Adjustment was made for age, cycle number and uterine length, uterine body AP, uterine transverse and uterine volume for fertility outcomes. For early pregnancy outcomes adjustment was made for age, gravidity, uterine length, uterine body AP, uterine transverse and uterine volume. For maternal and neonatal outcomes adjustment was made for age, uterine length, uterine body AP, uterine transverse uterine volume and parity given the association between some adverse pregnancy outcomes and advanced maternal age and parity (232, 328-330). To investigate predictive factors for spontaneous pregnancy in the POI cohort the regression model included previous pregnancy status, age at diagnosis, height, BMI, FRAXA status, menstrual history and thyroid status. Results were presented as Odds Ratio (OR) and 95% CI.

CHAPTER 3

STUDY 1 RESULTS

EXAMINATION OF THE REPRODUCIBILITY OF UTERINE DIMENSIONS USING TRANSABDOMINAL ULTRASONOGRAPHY COMPARED TO TRANSVAGINAL ULTRASONOGRAPHY AND MRI

The aim of this study was to explore and detail the reproducibility of TAUS and compare reproducibility between TAUS and TVUS and MRI.

Inter and Intraobserver reproducibility of transabdominal ultrasound

Interobserver reproducibility of TAUS parameters was assessed in 18 subjects who had had a transabdominal scan completed by both observer A and B. The mean age of the participants was 20.5 years (SD 6.8). The descriptive data for interobserver reproducibility of the uterine parameters can be seen in Table 3.1. There were no significant differences for total uterine length, uterine body AP, transverse, cervix AP or volume measurements and the Bland Altman plots can be seen in Figure 3.1 showing no systematic bias in these measurements. There were significant differences for uterine body length and cervix length when measured separately. The intraclass correlations (ICC) for total uterine length, uterine body AP, cervix AP, transverse and uterine volume were all greater than 0.9 (Figure 3.2).

Table 3.1. Table demonstrating mean difference, 95% limits of agreement and intraclass correlations for interobserver reproducibility of uterine parameters assessed by transabdominal scan

	Interobserver reproducibility			
	Mean difference (SD) (Observer A – Observer B)	**95% Limits of agreement**	**Paired T test**	**ICC (95% CI)**
Uterine body length (mm)	5.27 (4.6)	-3.8 - 14.3	<0.001	0.87 (0.13 - 0.96)
Uterine cervix length (mm)	-7.23 (4.1)	-15.4 - 0.9	<0.001	0.58 (0.22 - 0.87)
Total uterine length (mm)	-0.83 (4.9)	-10.5 - 8.8	0.48	0.97 (0.92 - 0.98)
Uterine Body AP (mm)	-0.61 (2.0)	-4.5 - 3.3	0.21	0.97 (0.93 - 0.99)
Cervix AP (mm)	0.0 (2.0)	-3.9 - 3.9	1.00	0.96 (0.90 - 0.98)
Transverse (mm)	-0.12 (4.5)	-8.9 - 8.6	0.91	0.94 (0.84 - 0.98)
Uterine volume (mls)	-2.00 (4.2)	-10.3 - 6.3	0.07	0.99 (0.96 - 0.99)

Figure 3.1. Bland Altman plots for interobserver variability of transabdominal scan

(A) total uterine length (mm), (B) uterine body anterior posterior (mm), (C) transverse (mm) and (D) uterine volume (mls). Dotted line represents mean difference and dashed lines the 95% limits of agreement.

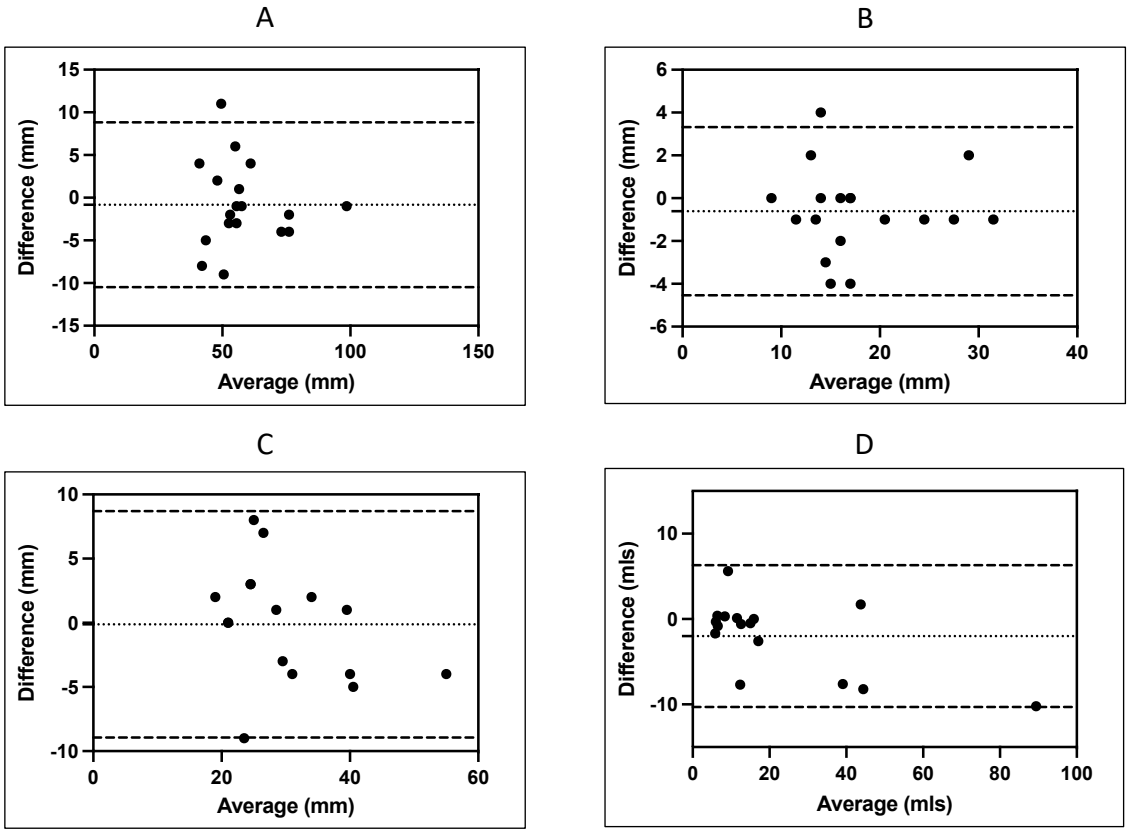
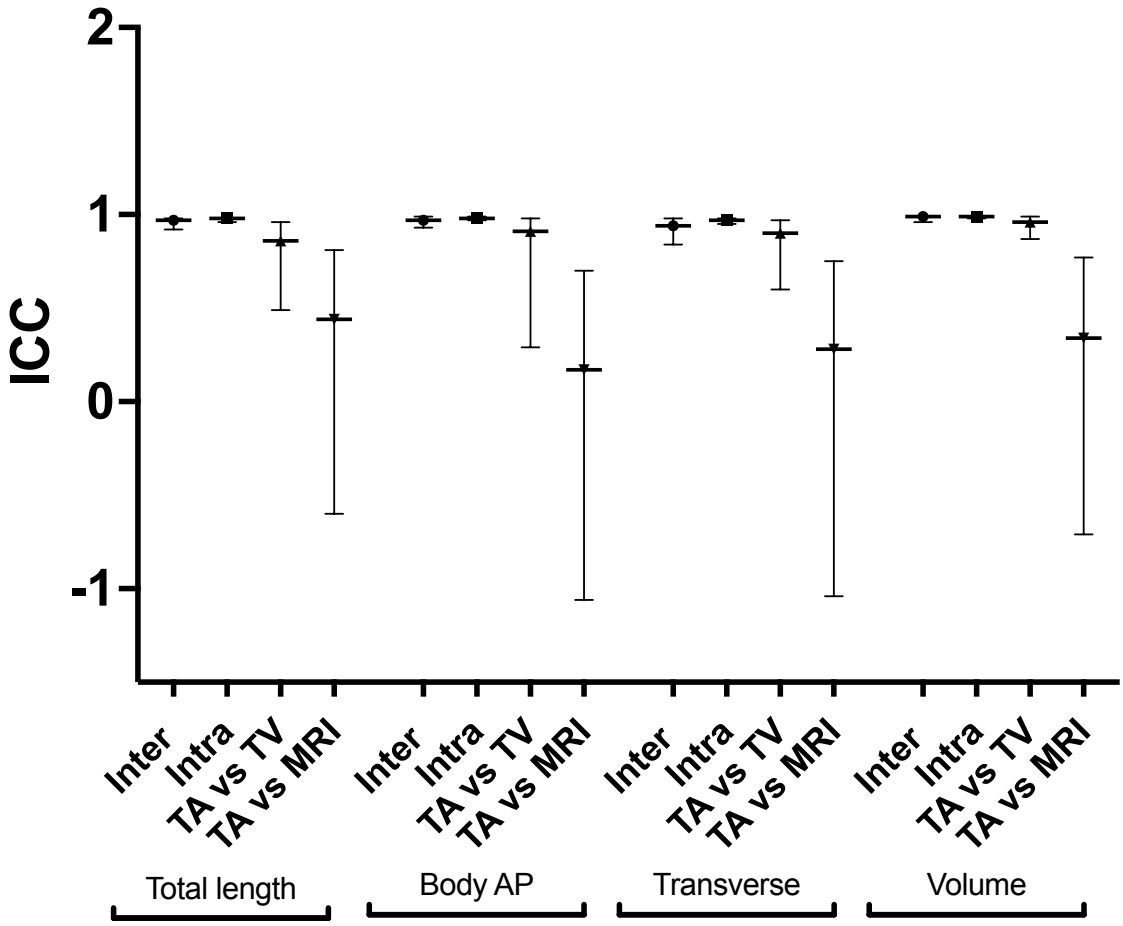


Figure 3.2. Graph demonstrating ICC for different uterine parameters



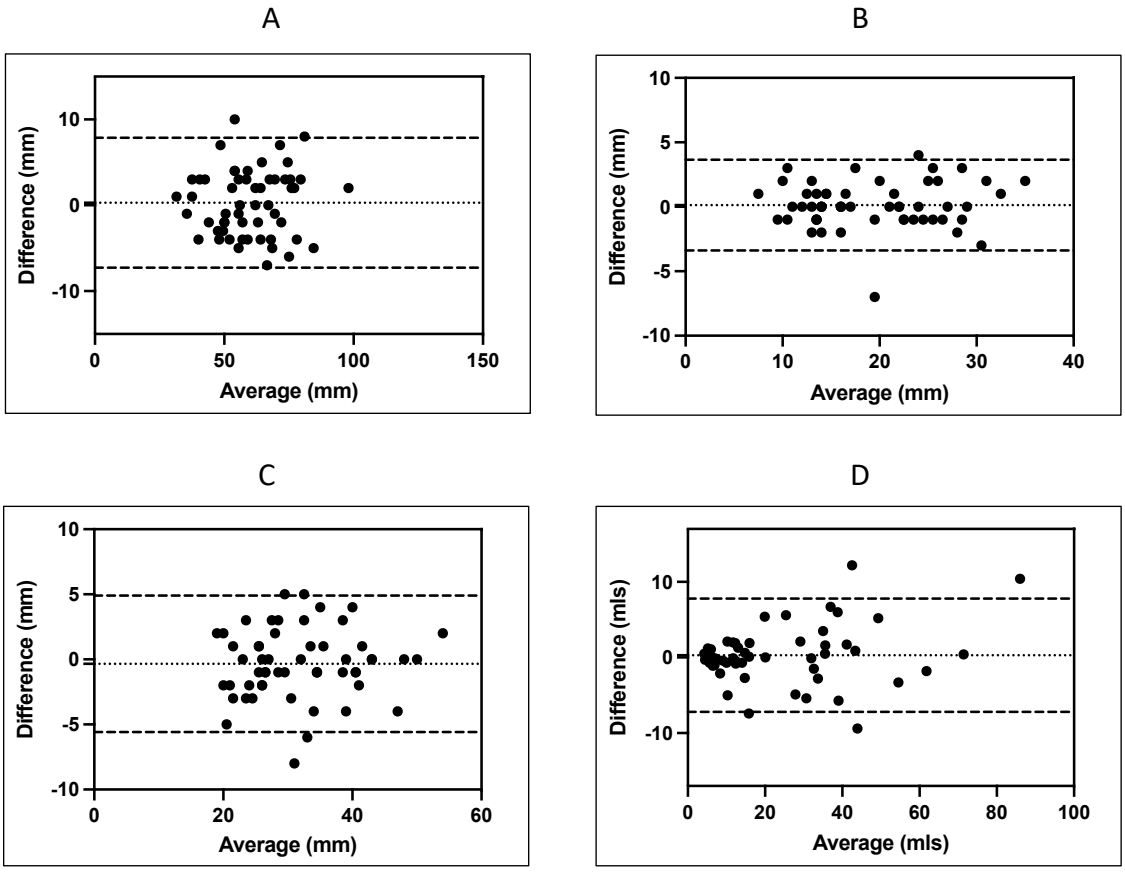
I tested intraobserver reproducibility of TAUS parameters by performing two scans in 55 participants (repeat scans completed within 30 minutes). The mean age of the participants was 19.3 years (SD 5.1) The descriptive data for intraobserver reproducibility of the uterine parameters can be seen in Table 3.2. There were no significant differences for all uterine parameters and the Bland Altman plots demonstrated no systematic bias in these measurements (Figure 3.3). The ICC for all parameters, with the exception of uterine cervix length, was greater than 0.9 (Figure 3.2).

Table 3.2. Table demonstrating mean difference, 95% limits of agreement and intraclass correlations for intraobserver reproducibility of uterine parameters assessed by transabdominal scan

	Intraobserver reproducibility			
	Mean difference (SD) (Observer A 1 – Observer A 2	**95% Limits of agreement**	**Paired T test**	**ICC (95% CI)**
Uterine body length (mm)	0.63 (4.3)	-7.8 - 9.1	0.28	0.95 (0.92-0.97)
Uterine cervix length (mm)	-0.34 (4.1)	-8.5 - 7.8	0.54	0.87 (0.78 - 0.92)
Total uterine length (mm)	0.29 (3.8)	-7.2 - 7.8	0.57	0.98 (0.96 - 0.98)
Uterine Body AP (mm)	0.12 (1.7)	-3.3 - 3.6	0.60	0.98 (0.97 - 0.99)
Cervix AP (mm)	-0.28 (2.3)	-4.8 - 4.3	0.38	0.95 (0.92 - 0.97)
Transverse (mm)	-0.34 (2.6)	-5.5 - 4.8	0.34	0.97 (0.95 - 0.98)
Uterine volume (mls)	0.28 (3.8)	-7.2 - 7.7	0.58	0.99 (0.98 - 0.99)

Figure 3.3. Bland Altman plots intraobserver variability of transabdominal scan

(A) total uterine length (mm), (B) uterine body anterior posterior (mm), (C) transverse (mm) and (D) uterine volume (mls). Dotted line represents mean difference and dashed lines the 95% limits of agreement.



Agreement between transabdominal and transvaginal scan modalities

I performed a TAUS followed by a TVUS in 10 women on the same clinic visit. The mean age of participants was 26.5 years (SD 2.1). The descriptive data for the uterine parameters can be seen in Table 3.3. There were no significant differences for total uterine length, transverse or uterine volume measurements between TAUS and TVUS however there were differences for uterine body length and AP and cervix length and AP measurements. Compared to TVUS, the uterine body length and cervix AP measurements were consistently greater when measured with TAUS, the latter may be explained due to the compression with the vaginal probe. The cervix length and uterine body AP measurements were

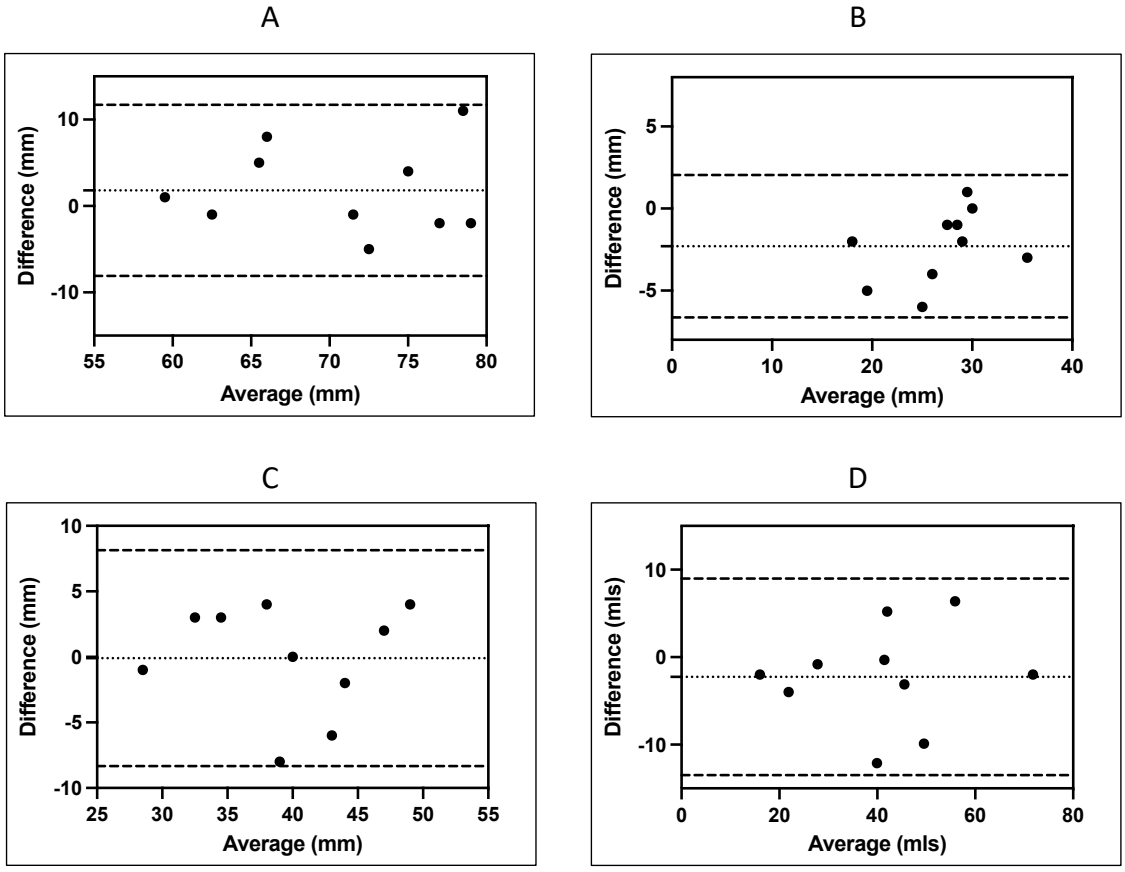
less when measured with TAUS and the latter may be secondary to bladder compression. The Bland Altman plots can be seen in Figure 3.4. Whilst the ICC for total uterine length, uterine body AP and transverse were above 0.9, the 95% CI were wide suggesting that the agreement between the modalities may not be reliable (Figure 3.2). The uterine volume ICC was 0.96 (95% CI 0.87 – 0.99).

Table 3.3. Table demonstrating mean difference, 95% limits of agreement and intraclass correlations of uterine measurements between transabdominal and transvaginal scans

	TAUS vs TVUS			
	Mean difference (SD) (TA – TV)	**95% Limits of agreement**	**Paired T test**	**ICC (95% CI)**
Uterine body length (mm)	4.7 (3.1)	-1.5 - 10.9	0.001	0.78 (-0.21 - 0.95)
Uterine cervix length (mm)	-2.7 (3.1)	-8.9 - 3.5	0.02	0.71 (-0.07 - 0.92)
Total uterine length (mm)	1.8 (5.0)	-8.1 - 11.7	0.28	0.86 (0.49 - 0.96)
Uterine Body AP (mm)	-2.3 (2.2)	-6.6 - 2.0	0.009	0.91 (0.29 - 0.98)
Cervix AP (mm)	2.7 (3.1)	-3.5 - 8.9	0.02	0.50 (-0.36 - 0.85)
Transverse (mm)	-0.1 (4.2)	-8.3 - 8.1	0.94	0.90 (0.60 - 0.97)
Uterine volume (mls)	-2.2 (5.7)	-13.4 - 8.9	0.24	0.96 (0.87 - 0.99)

Figure 3.4. Bland Altman plots for agreement between TAUS and TVUS

(A) total uterine length (mm), (B) uterine body anterior posterior (mm), (C) transverse (mm) and (D) uterine volume (mls). Dotted line represents mean difference and dashed lines the 95% limits of agreement



Agreement between transabdominal and MRI scan modalities

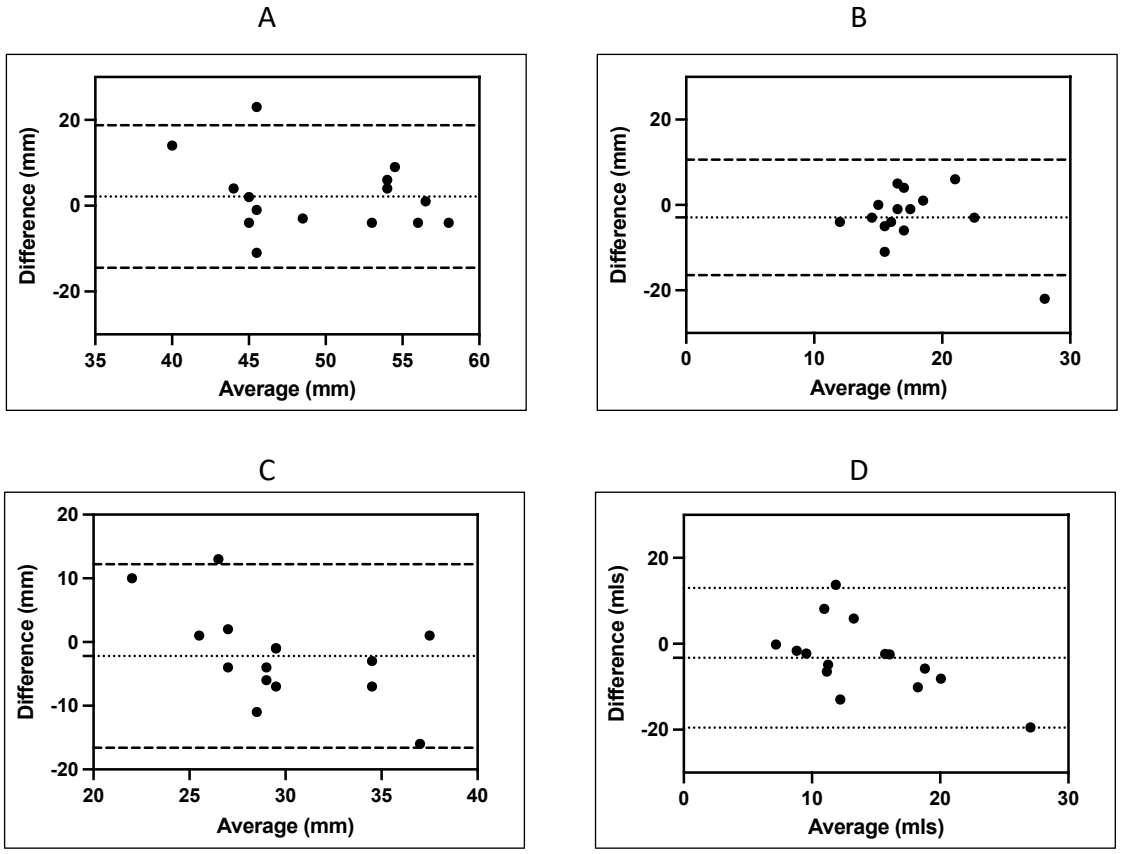
Fifteen participants underwent both a TAUS and MRI assessment of uterine dimensions. The mean age of the participants was 15.8 years (SD 1.9). The descriptive data for the uterine parameters can be seen in Table 3.4. For all uterine dimensions, although there were no significant differences, there was very poor ICC between US and MRI for all measurements (Figure 3.2). The Bland Altman plots can be seen in Figure 3.5.

Table 3.4. Table demonstrating mean difference, 95% limits of agreement and intraclass correlations of uterine measurements between transabdominal and transvaginal modality and transabdominal scan and MRI

	TAUS vs MRI			
	Mean difference (SD) (Ultrasound – MRI)	**95% Limits of agreement**	**Paired T test**	**ICC (95% CI)**
Total uterine length (mm)	2.1 (8.4)	-14.4 - 18.5	0.34	0.44 (-0.6 - 0.81)
Uterine Body AP (mm)	-2.9 (6.9)	-16.4 - 10.5	0.12	0.17 (-1.06 - 0.70)
Transverse (mm)	-2.2 (7.3)	-16.6 - 12.2	0.26	0.28 (-1.04 - 0.75)
Uterine volume (mls)	-3.2 (8.2)	-19.5 - 12.9	0.14	0.34 (-0.71 - 0.77)

Figure 3.5. Bland Altman plots for agreement between TAUS and MRI

(A) total uterine length (mm), (B) uterine body anterior posterior (mm), (C) transverse (mm) and (D) uterine volume (mls). Dotted line represents mean difference and dashed lines the 95% limits of agreement.



Summary

In summary, there was no significant difference for uterine length, uterine body anterior posterior, transverse and uterine volume measurements when assessing interobserver and intraobserver reproducibility of TAUS and the intraclass correlations (ICC) were >0.9 for all parameters. There was generally poor ICC with greater variability for the comparison of TAUS and TVUS and poor ICC between TAUS and MRI.

CHAPTER 4

STUDY 2 RESULTS

CHALLENGES IN DEVELOPING A QUANTITATIVE METHOD OF MEASURING BREAST DEVELOPMENT USING 3D IMAGING - AN EXAMPLE OF A NOVEL METHOD FOR USE IN INDUCED BREAST DEVELOPMENT WITH EXOGENOUS OESTROGEN

The aim of this study was to develop a novel technique using 3D imaging to determine change in breast volume that is applicable when no pre-existing breast contours are present. This would permit longitudinal quantitative assessment of breast differential volume during pubertal induction. I also examined the feasibility and reproducibility of anatomical landmarking.

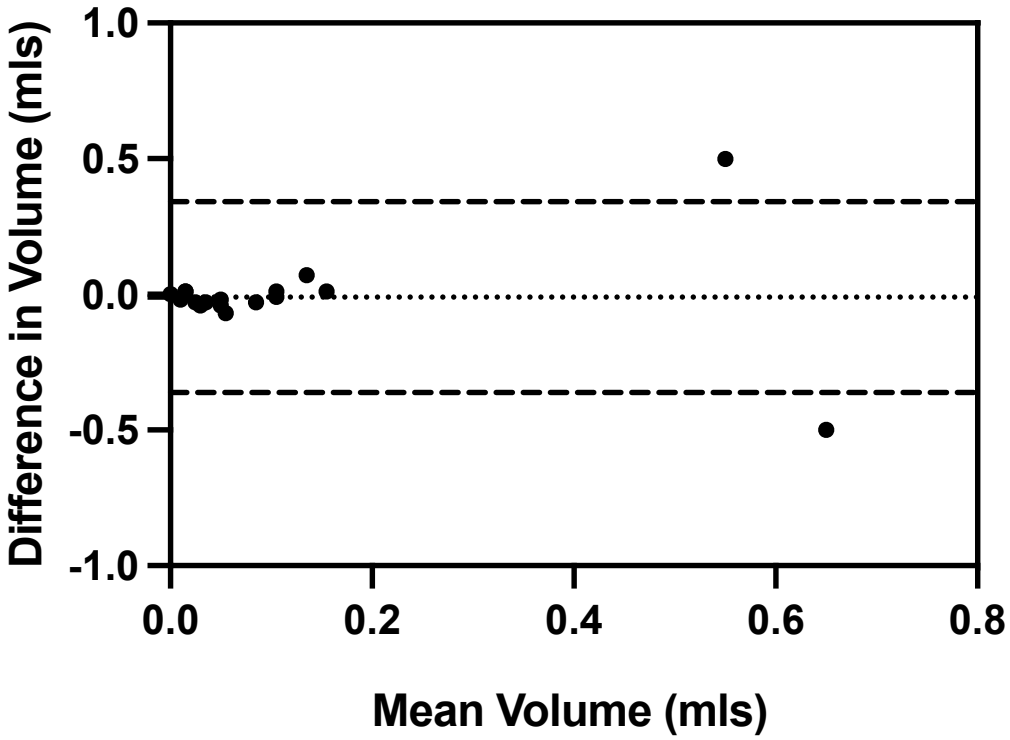
Static breast imaging and intraobserver variability

To ensure that the breast volume change was not secondary to movement or artefact I calculated the mean differential volume using 17 different combinations of 9 images of the same male participant taken on different days. No volume change in the breast region was expected. The mean differential volume calculated was minimal; on the first round of alignment it was 0.11mls (SD 0.20) and on the second round 0.12 mls (SD 0.21). Examining intraobserver reproducibly, the mean difference between the 2 measurements was – 0.01mls with Bland- Altman limits of agreement of - 0.36 - 0.34mls p = 0.84 (Figure 4.1) suggesting that there was no systematic bias. Visually examining the Bland Altman plot, there appeared to be two outliers, where slightly larger breast volumes were calculated for the male participant, however the mean volumes were still very minimal and the difference between measurements remained

acceptably small at 0.5 – 0.6 mls. The intraclass correlation analysis between the 2 measurements was 0.77 (95% CI 0.37 -0.92) suggesting good reliability.

Figure 4.1. Bland Altman plot for intraobserver variability of breast volume calculation on the male participant static images

The dotted line represents the mean differences between the repeated values and the dashed lines represents the 95% limits of agreement.



Comparison to reference warped image volume

I compared the volume change produced by image warping to that calculated by operator alignment (EB). The descriptive data comparing the reference warped volume to the operator aligned volumes are shown in Table 4.1.

The known volume produced by 5mm warping of image A was 52.3mls. The volumes calculated using the warped image A to the other static images varied +/- 8.1mls and 7.2mls. The known volume generated by 5mm warping of image D was 44.2mls and the volumes calculated when superimposed with the other static images varied +/- 5.4mls and 4.8mls. The known volume produced by

10mm warping of image A was 102.4mls and the volumes calculated with static image alignment varied +/- 8.3mls and 7.5mls. The known volume produced by 10mm of image D was 89.3mls and the volumes calculated with static images varied +/- 5.8mls and 13.4mls. The one-way T tests demonstrated that there was no significant difference between the known volumes generated by computer image warping and the volumes calculated by image alignment of the static images to the warped image.

Table 4.1. Table demonstrating the descriptive data comparing the reference computer-generated volume to the operator aligned volumes

The reference breast volume (mls) produced by thin warp slicing of static image A and image D (both 5 and 10mm). The minimal, maximum, mean differential volume and mean difference is shown when the warped image is superimposed with the 8 other static images compared to the reference volume. P values are displayed demonstrating the difference between the reference volume and that calculated by image alignment with static images.

	Reference volume (mls)	Minimum volume (mls)	Maximum volume (mls)	Mean volume (SD)	Mean difference (SD)	P value
Image A 5mm displacement	52.3	45.1	60.4	52.7 (5.4)	-0.37 (5.4)	0.85
Image D 5mm displacement	44.2	39.4	49.6	43.1 (3.3)	1.06 (3.3)	0.40
Image A 10mm displacement	102.4	94.9	110.7	100.8 (5.0)	1.62 (5.0)	0.39
Image D 10mm displacement	89.3	75.9	95.1	84.9 (6.1)	4.4 (6.1)	0.08

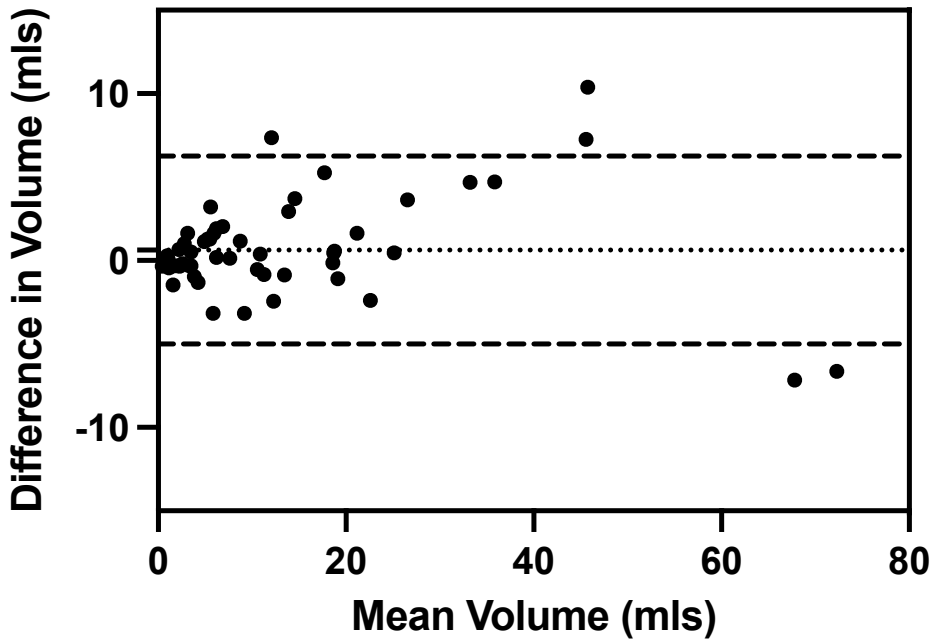
Application in participants undergoing induced breast development

Intraobserver reproducibility of breast volume calculation was assessed using 58 images of participants undergoing treatment with exogenous oestradiol.

The mean difference between the two measurements was 0.62mls with Bland-Altman limits of agreement of -5.00 - 6.25 p = 0.10 (Figure 4.2) demonstrating no systematic bias. Visually inspecting the Bland Altman plot, it appears that the variation in measurements between the two calculated volumes was minimal in the majority. As the breast volume increased so did the differences in measurements, however the difference was still relatively small. The intraclass correlation analysis between the 2 measurements was 0.99 (95% CI 0.98 - 0.99) suggesting excellent reliability.

Figure 4.2. Bland Altman plot for intraobserver variability of breast volume on participants undergoing induced breast development

The dotted line represents the mean differences between the repeated values and the dashed lines represents the 95% limits of agreement.



Reproducibility of landmarking

A total of 14 participants underwent breast imaging for the purpose of examining the reproducibility of landmarking. The difference in placement of the markers on the 7 anatomical landmarks for the 3 techniques is shown in Table 5.2. There was significant difference in all markers with all different techniques tried. The smallest differences were observed for the nipple marker placement, whereas the marker placement on the anterior axillary line had the largest difference.

Table 4.2. Table demonstrating the mean difference between placement of markers on the 7 anatomical landmarks. The 3 different techniques trialled are shown.

The P values are displayed demonstrating the difference from zero.

	Static		Dynamic		Colour contour	
	Mean Difference (mm) (SD)	P value	Mean Difference (mm) (SD)	P value	Mean Difference (mm) (SD)	P value
Nipple	1.6 (1.1)	<0.001	1.17 (0.7)	<0.001	2.5 (3.7)	0.03
Lateral breast	11.5 (5.8)	<0.001	11.8 (7.4)	<0.001	12.7 (7.1)	<0.001
Medial breast	8.0 (6.1)	<0.01	7.1 (3.8)	<0.001	13.8 (6.3)	<0.001
Infra mammary fold	7.2 (7.1)	0.002	6.1 (3.7)	<0.001	6.1 (4.1)	<0.001
Superior breast	6.9 (4.4)	<0.01	8.0 (5.1)	<0.001	10.7 (12.4)	0.01
Sternal notch	5.8 (5.7)	0.002	6.7 (4.1)	<0.001	6.9 (3.7)	<0.001
Anterior axillary line	12.0 (9.4)	<0.001	17.0 (12.4)	<0.001	7.8 (5.8)	<0.01

Summary

In summary good intraobserver reproducibility (intraclass correlation (ICC) 0.77) was demonstrated with static image manipulation. Validity of the imaging technique was established as there was no significant difference between the known reference volume produced by computer generated warping and that calculated by manual image manipulation. There was excellent intraobserver reproducibility for breast volume calculation in participants undergoing induced breast development (ICC 0.99). There was poor reproducibility for landmarking for all different techniques trialled.

CHAPTER 5

STUDY 3 RESULTS

UTERINE VOLUME AFTER INDUCTION OF PUBERTY IN WOMEN WITH HYPOGONADISM

The aim of this study was to assess uterine size using ultrasound in those who had undergone pubertal induction treatment compared to a reference group who had experienced spontaneous puberty. I also explored factors which may influence uterine size.

Ninety-five women with hypogonadism were recruited: 48 women with TS, 32 with POI and 15 with GD (Table 5.1). Women with TS had a variety of karyotypes, 22 with 45,X; 5 with mosaic 45,X/46,XY; 11 with isochromosome Xq; 3 with mosaic 45,X/46,X,r(X); 4 with partial X deletion; 1 with mosaic 45,X/46,XX and 1 with mosaic 45,X/47,XXX. The gonadotrophin deficiency group comprised 2 women with hypothalamic amenorrhoea, 4 with hypopituitarism and 9 with hypogonadotrophic hypogonadism. Thirty-five nulliparous women with spontaneous menstrual cycles formed the reference group.

Table 5.1. Participant characteristics and uterine parameters in women with hypogonadism and reference groups

Results displayed as median and 5th and 95th centiles. [a] Statistically different from POI and GH; [b] Statistically different from those with hypogonadism; [c] Statistically different from the reference group.

	Turner Syndrome	Premature Ovarian Insufficiency	Gonadotrophin Deficiency	All Hypogonadism Combined	Reference Group
n	**48**	**32**	**15**	**95**	**35**
Age (years)	25 (16 - 42.1)	25.5 (18 - 37.3)	24 (16 - 28)	24 (16.8 - 40)	34 [b] (26 - 38.2)
Height (cm)	151 [a] (138 - 164.4)	165 (136.5 -183)	163 (150 - 167)	155 (140.3 - 173.8)	164.7 [b] (150.7 - 74.4)
Weight (kg)	63 (45.1 - 94.7)	66.5 (43.2 - 121.2)	62.5 (44 -130)	63.7 (44.3 - 110.9)	62.5 (46.4 - 87.6)
BMI (kg/m^2)	27.6 [a] (18.2 - 39.8)	23.7 (18.3 - 37.7)	22.1 (16.4 - 50)	25.2 (18.1 - 39.9)	23 [b] (19.3 - 30)
Age at Presentation (years)	7.5 [a] (0 - 19.3)	16 (11.2 - 17.9)	17 (3 - 21)	14 (0 - 19)	n/a
Age Starting E2 (years)	13 [a] (10 - 16.6)	16 (12.2 - 18.4)	17 (11 - 22)	15 (11 - 18.4)	n/a
Menarche Age (years)	15 [a] (13 - 18)	17 (13.5 - 20.4)	18 (13 - 23)	16 (13 - 21)	13 [b] (10.6 - 16)
Total Uterine Length (mm)	63 (43 - 89)	67.5 (44 - 83)	62 (41 - 78)	64 [c] (43 - 83)	71 (57.6 - 85.2)

	Turner Syndrome	Premature Ovarian Insufficiency	Gonadotrophin Deficiency	All Hypogonadism Combined	Reference Group
Anterior Posterior Uterine Body (mm)	24 (13.3 - 32.5)	25 (12.6 - 33.7)	26 (13 - 30)	24 [c] (13 - 32.2)	31 (24 - 38)
Transverse (mm)	36 (21.4 - 52.1)	34.5 (25.6 - 46.3)	36 (18 - 47)	35 [c] (23.6 - 50.2)	40 (33.8 - 51.4)
Volume (cm^3)	28.2 (8.2 - 76.4)	29.3 (6.9 - 64.3)	28.2 (5 - 57.1)	28.9 [c] (7.7 - 69.2)	43.9 (28.5 - 78.4)
Fundal Cervical AP Ratio	1.19 (0.95 - 1.97)	1.23 (0.77 - 1 .64)	1.29 (0.90 - 1.65)	1.22 [c] (0.92 - 1.67)	1.39 (1.07 - 2.13)

There was no difference in age or weight across the diagnostic groups, however women with TS were significantly shorter and had a higher BMI than the other diagnostic groups (Height TS vs POI p = 0.00 and TS vs GD p = 0.02; BMI TS vs POI p = 0.03 and TS vs GD p = 0.04). Women in the reference group were significantly taller and had a lower BMI than those in the diagnostic groups however further analysis revealed that this was only significant between those with TS and the reference group (Height p = 0.03; BMI p = 0.04). I however could not identify correlation between height or weight and uterine parameters in the reference group. Women in the reference group were significantly older than those in the diagnostic groups (p = <0.001), however I could not identify any correlation between age and uterine parameters in the reference group. Those with TS presented significantly earlier, started oestradiol therapy and achieved induced menarche significantly earlier than the other diagnostic groups (Presentation age TS vs POI p = <0.001 and TS vs GD p = 0.04; Oestradiol initiation age TS vs POI p = <0.001 and TS vs GD p = 0.01; Menarche age TS vs POI p = 0.01 and TS vs GD p = <0.001). Subjects in the reference group had significantly earlier menarche than those in the diagnostic groups (p = <0.001) however I could not identify any correlation between age of menarche and uterine parameters in the reference group.

Across the diagnostic groups, 83.7% of women reported little or no breast development (up to Tanner stage 2) prior to the initiation of oestrogen therapy. Those with GD and POI had 25.8% and 26.7% of prior breast development respectively compared to 6.5% of women with TS (p = 0.04). Uterine size was not predicted by current age, prior breast development (i.e. prior oestrogen exposure), height, weight, age of presentation, start age of oestrogen therapy, duration of unopposed oestradiol therapy, age at menarche, or timing of the scan

relative to the hormone cycle and scanning modality (transvaginal or transabdominal). Of the 48 women with TS, 9 did not receive GH, however the use of GH did not have any significant effect on uterine parameters nor final height.

The summary of uterine parameters for the diagnostic groups and the reference group is summarised in Table 5.1. Women with hypogonadism had significantly shorter uterine length ($p = 0.02$) and reduced uterine volume ($p = <0.001$) compared to the reference group despite being on full adult oestrogen replacement. All women in the reference group attained a mature uterine configuration with an FCR >1, compared to 84% of those with hypogonadism ($p = 0.01$). There were no differences in ultrasound measurements between the diagnostic groups. Figure 5.1 and Figure 5.2 demonstrate that 24% and 48% of the diagnostic group had total uterine length and uterine volume measurements less than the 5^{th} percentile of the reference group respectively.

Figure 5.1. Scatterplot of total uterine length in women with hypogonadism taking maintenance oestrogen replacement compared to reference group who had experienced normal spontaneous puberty.

Twenty four percent of women with hypogonadism had uterine length below the 5th centile for the reference group (dotted line).

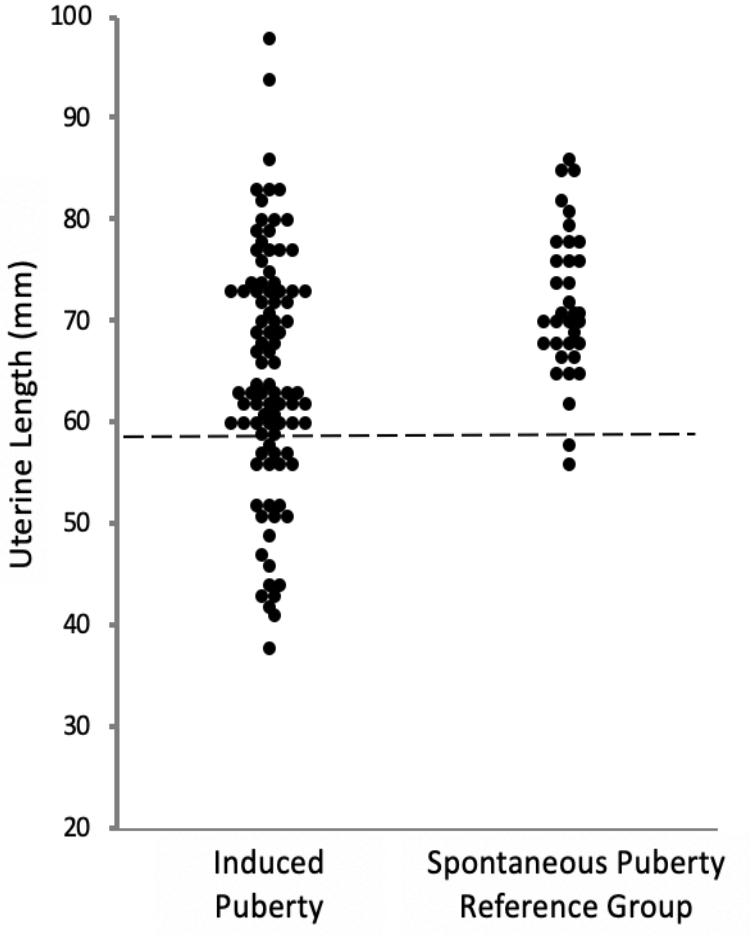
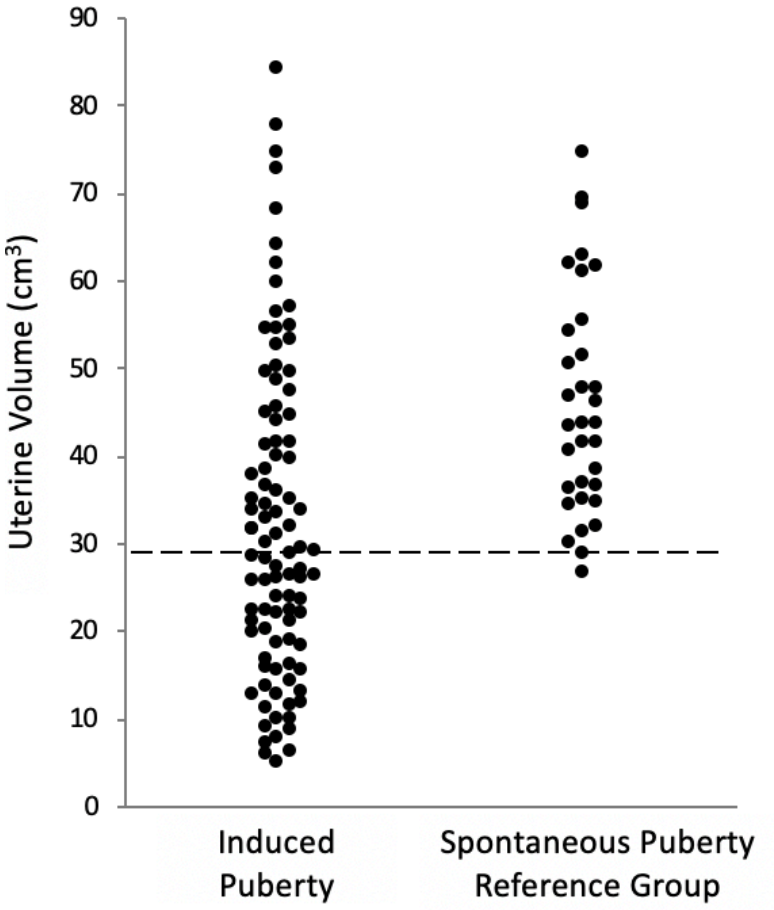


Figure 5.2. Scatterplot of uterine volume in women with hypogonadism taking maintenance oestrogen replacement compared to reference group who had experienced normal spontaneous puberty

Forty eight percent of women with hypogonadism had uterine volume below the 5th centile for the reference group (dotted line).



I assessed whether the mode of induction of puberty was a determinant of adult uterine size. Induction of puberty was initiated with a range of different oestrogen preparations which were categorised into 3 groups: unopposed low start dose ethinylestradiol (57%), transdermal oestradiol (21%) and oral contraceptive pill (17%). The remaining 5% had received oral 17β oestradiol, conjugated equine oestrogen or an HRT preparation. There was no difference in uterine size between the oestrogen groups as summarised in Table 5.2.

Table 5.2. Uterine size parameters comparing different oestrogen preparations used for induction of puberty

Uterine Parameter	Oral Contraceptive Pill	Transdermal Patch	Unopposed Ethinylestradiol low dose
n	**15**	**19**	**51**
Total Length (mm)	69 (42 - 78)	69 (56 - 80)	63 (42.8 - 84.2)
Anterior Posterior Body (mm)	22 (13 - 31)	26 (16 - 31)	24 (12.6 - 34.6)
Transverse (mm)	33 (17 - 53)	36 (25 - 43)	35 (21.6 - 47.4)
Volume (cm^3)	25.7 (10.1 - 68.3)	31.2 (6.2 - 52.7)	28.7 (6.8 - 73.5)

Uterine measurements were compared in subgroups defined by the type of HRT being used. The content of maintenance HRT preparations included 69 (73%) women using 17β oestradiol, 18 (19%) using ethinylestradiol and 8 (8%) using conjugated equine oestrogen. In order to explore the possible effect of the current dose of oestrogen on uterine size I categorised the various HRT preparations as follows accepting that dose equivalents are approximate: Low dose (ethinylestradiol ≤ 10mcg and oral 17β oestradiol < 2mg) medium dose (ethinylestradiol 20mcg, oral 17β oestradiol 2 mg, transdermal oestradiol patch 50mcg and conjugated equine oestrogen 0.625mg) and high dose; (ethinylestradiol ≥30mcg, oral 17β oestradiol >2mg, transdermal oestradiol patch >50mcg and conjugated equine oestrogen 1.25mg). 11 (12%) were taking low dose, 68 (71%) medium dose and 16 (17%) high dose. Based on this categorisation, neither the type of oestradiol nor dose of HRT affected uterine size parameters.

For those taking oestradiol preparations, the serum concentrations were available in 22 women and this parameter was positively correlated with all uterine parameters (total uterine length r = 0.549, anterior posterior r = 0.485, transverse r = 0.445 and volume r = 0.551).

Summary

In summary, those with hypogonadism and induced puberty had significantly reduced uterine dimensions compared to the reference group (uterine length 64 mm vs 71mm p = 0.02, uterine volume 28.9ml vs 43.9ml p = < 0.001). All women in the reference group attained a mature uterine configuration with an FCR >1, compared to 84% of those with hypogonadism (p = 0.01). 24% and 48 % of the diagnostic group had total uterine length and uterine volume measurements less than the 5^{th} percentile of the reference group respectively. In a subgroup of 22 women in whom serum oestradiol concentrations could be analysed, there was a positive correlation between this parameter and uterine volume.

CHAPTER 6

STUDY 4 RESULTS

VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM.

Induction of puberty with exogenous oestrogen results in considerable variability in final uterine and breast volumes and therefore I set out to quantify the variability of these two outcome measures with a view to establishing monitoring methods that could be used to individualise treatment protocols.

Sixteen females were recruited, 7 with hypogonadotrophic hypogonadism (HH), 6 with premature ovarian insufficiency (POI), 2 with Turner syndrome (TS) and 1 with 17 beta hydroxylase deficiency. The median age at time of initiation of therapy was 16.5 years (5^{th} and 95^{th} percentile 11 -17 years). Only 2 participants were under the age of 14 at diagnosis and treatment initiation. The anthropometric, hormonal, bone density and Tanner breast staging and uterine parameters at baseline and 8 months are presented in Tables 6.1 and 6.2.

Individual variation in the response to oestrogen was assessed over the first 8 months of set dose protocol. All participants completed 8 months of treatment. There was a three-fold difference in the minimum and maximum change in sE2 and a 1.5-fold difference in these values for uterine volume, uterine length and transverse measurements. There was a seventeen-fold difference in the differential breast volume change. Furthermore, there were fluctuations apparent between each time point rather than a linear change. The change in uterine,

oestradiol and breast volume measurements are shown in Tables 6.1 and 6.2 and Figures 6.1 and 6.2.

Table 6.1. Anthropometric and hormonal data in participants undergoing pubertal induction at baseline and 8 months treatment

Results are displayed as mean and 95% CI. P values are displayed for the differences between baseline and 8 months.

	Baseline	8 months	p Value
Height z score	- 0.49 (-1.36 - 0.36)	-0.19 (-0.98 - 0.59)	0.001
Height velocity cm/year		3.41 (1.71 - 5.10)	
Weight (kg)	57.0 (48.5 - 65.6)	58.2 (49.9 - 66.5)	0.56
BMI (kg/m^2)	22.3 (20.2 - 24.4)	22.2 (19.9 - 24.5)	0.86
Hip circumference (cm)	91.4 (85.5 - 97.3)	92.9 (86.7 - 99.1)	0.37
Waist circumference (cm)	78 (71.4 - 84.6)	76.1 (71.3 - 80.8)	0.44
Hip waist ratio	0.85 (0.81 - 0.89)	0.82 (0.79 - 0.84)	0.10
Total body fat (%)	31.8 (27.1 - 36.4)	29.3 (24 - 34.7)	0.13
sE2 (pmol/L)	11.7 (9.6 - 13.9)	80.2 (51.6 - 108.9)	<0.001
FSH (IU/L)	93.2 (68.1 - 118.4)	44.2 (19 - 69.4)	0.01
LH (IU/L)	30.8 (23 - 38.7)	21.3 (9.4 - 33.3)	0.17

Table 6.2. Uterine and breast data in participants undergoing pubertal induction at baseline and 8 months

Results for uterine parameters are displayed as mean and 95% CI at baseline and at 8 months. Tanner breast stage at baseline and 8 months is displayed as percentage. Breast differential volume at 8 months is displayed as median and 5th and 95th centile. P values are displayed for the differences between baseline and 8 months.

	Baseline	8 months	p Value
Total Uterine Length (mm)	33.1 (28.8 - 37.4)	50.8 (47.6 - 53.9)	<0.001
AP Uterine Body (mm)	6.7 (5.5 - 7.9)	16.3 (14.2 - 18.4)	<0.001
AP Cervix (mm)	6.5 (5.4 - 7.6)	12.4 (10.7 - 14.1)	<0.001
Fundus AP: Cervix AP ratio (FCR)	1.0 (0.9 - 1.1)	1.3 (1.1 - 1.5)	0.001
Transverse (mm)	16.8 (14.2 - 19.4)	28.7 (26.7 - 30.7)	<0.001
Uterine Volume (ml)	2.2 (1.3 - 3.1)	12.5 (10.3 - 14.7)	<0.001
Tanner breast stage n (%)	B1: 8 (50%) B2: 8 (50%)	B1: 1 (6.3%) B2: 7 (43.8%) B3: 6 (37.5%) B4: 2 (12.5%)	0.001
Breast Differential Volume (ml)		9.52 (1.75 - 101.2)	

Figure 6.1. Graphs demonstrating changes in uterine transverse diameter, uterine length and serum oestradiol concentrations for each subject every 2 months for the total of 8 months

Dashed line represents mean for each time point.

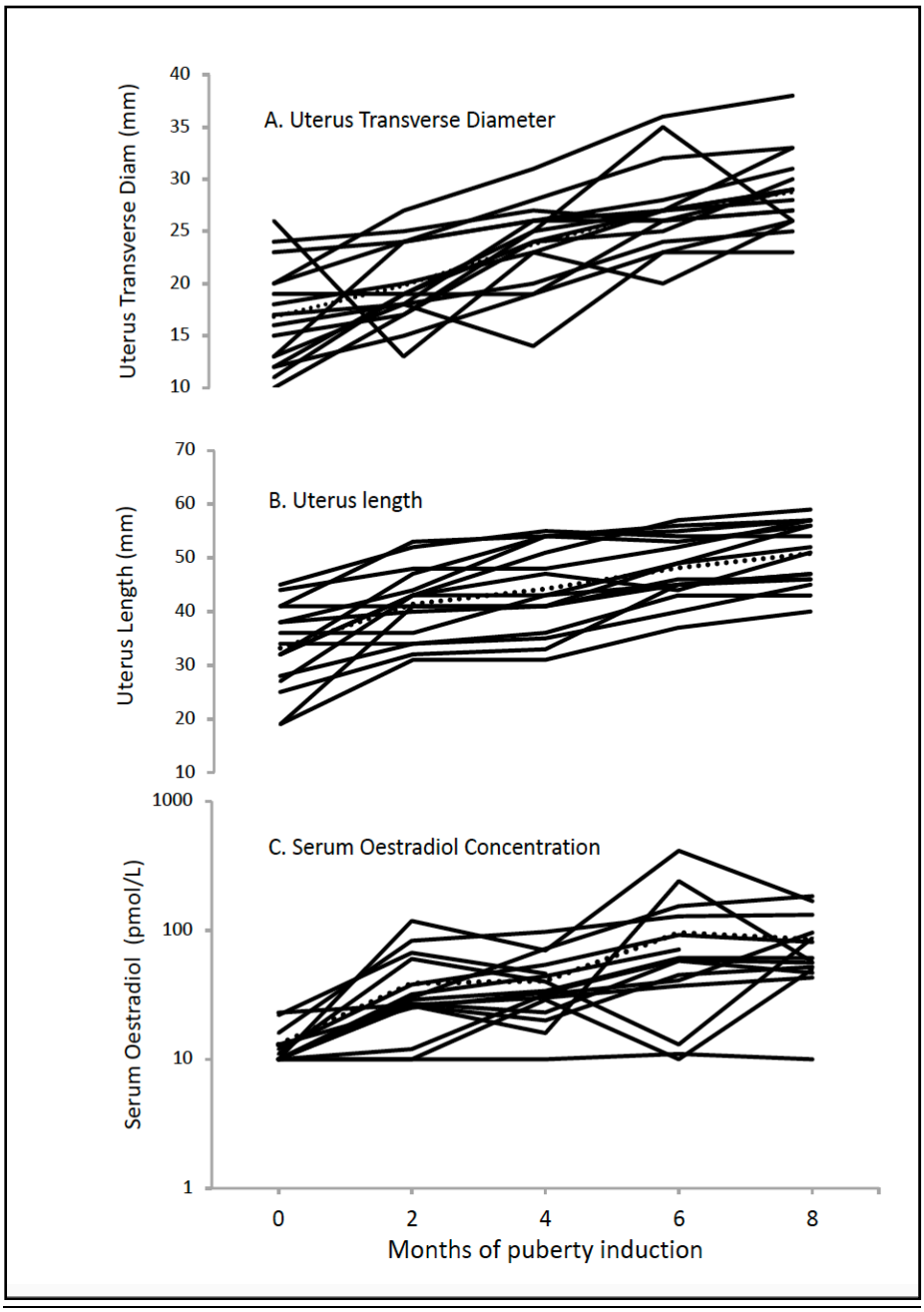
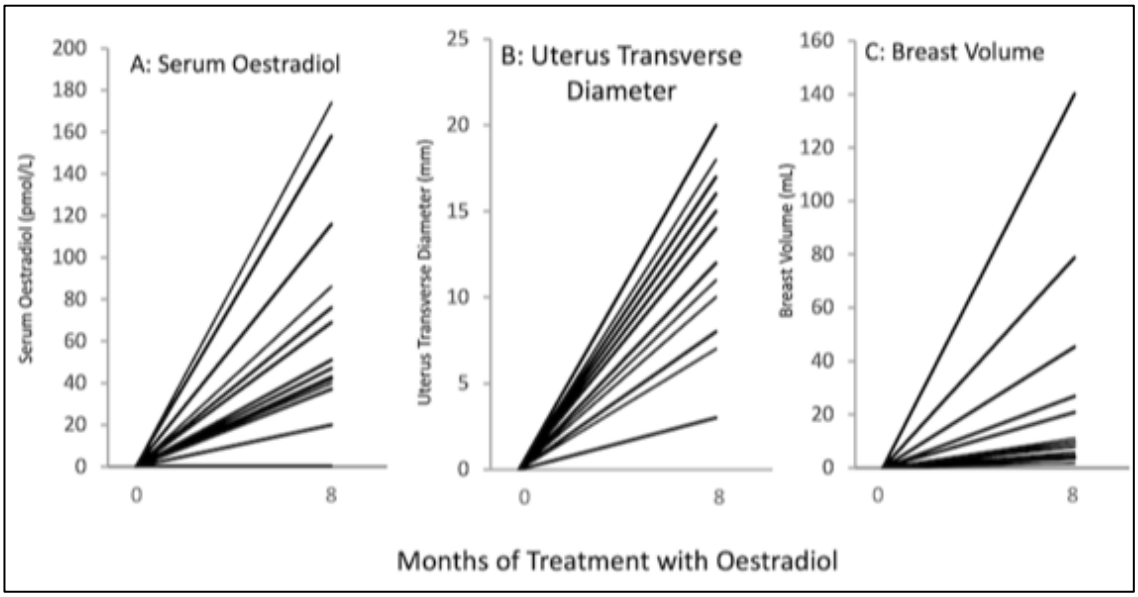


Figure 6.2. Graphs demonstrating absolute change in serum oestradiol concentrations, uterine transverse diameter and breast volume for each subject between treatment initiation and 8 months



Anthropometric measurements

Anthropometric data is shown in Table 6.1. There was no difference in mean weight, BMI, hip circumference, waist circumference, HWR and total body fat between baseline and after 8 months of treatment. Height z score was the only parameter to show significant change over the treatment period. 13/16 (81.2%) participants increased in height over the 8-month study period and 3 did not change in height. Height increase varied from 1 to 8cm. Markers of adiposity demonstrated no clear pattern during treatment time.

Hormonal profile

Oestradiol concentrations were available for all females at baseline and 14/16 at 8 months. At baseline, the E2 concentration was below the limit of detection of 10 pmol/L in 10/16 subjects. There was a significant rise of E2 after 8 months of treatment, and the mean increase in E2 concentration was 68.5 pmol/L (95% CI 39.5 - 97.4pmol/L). At 8 months, the E2 concentration remained <10pmol/L in 1

subject. This participant was not removed from the analysis as there was no obvious deviation from the protocol justifying exclusion.

The 8 subjects with raised gonadotrophins at baseline showed significant decline in the mean FSH ($p = 0.01$) but not LH after 8 months of treatment. FSH concentrations were suppressed by a mean of -49.0 IU/L (95% CI -14.6 - -83.4 IU/L).

The intraclass correlation (ICC) for standard assay and sensitive assay for oestradiol concentrations at 8 months was 0.91 (95% CI 0.72- 0.97) suggesting good reliability between the two assays.

Bone density data

The changes in Z score, BMD and BMC in both the total hip and lumbar spine from baseline to 8 months can be seen in Table 6.3. There were significant changes in lumbar spine measurements but not hip measurements with 8 months of treatment. There was no association between bone density measurements and oestradiol concentration at 8 months.

Table 6.3. Bone density data in participants undergoing pubertal induction at baseline and 8 months

Results are displayed as mean and 95% CI at baseline and at 8 months. P values are displayed for the differences between baseline and 8 months.

	Baseline	8 Months	P Value
Total Hip Z score	-1.6 (-2.3 - -0.9)	-1.6 (-2.2- -1.0)	0.30
Total Hip BMC (g)	26.5 (22 - 31)	26.3 (21.8 - 30.8)	0.16
Total Hip BMD (g/cm^2)	0.74 (0.67 - 0.82)	0.74 (0.67 - 0.81)	0.48
Lumbar spine Z score	-3.2 (-3.8 - -2.5)	- 2.9 (-3.6 - - 2.2)	0.02
Lumbar spine BMC (g)	32.7 (28.9 - 36.5)	34.9 (31.2 - 38.6)	<0.001
Lumbar spine BMD (g/cm^2)	0.68 (0.63 - 0.72)	0.71 (0.66 - 0.75)	0.006

Uterine growth - Ultrasound

There was significant growth in all uterine parameters with 8 months of treatment. The mean increase in uterine length was 17.6mm (95% CI 14.4 - to 20.7 mm), the mean increase in transverse diameter was 11.9mm (95% CI 9.1 - 14.7mm) and the mean change in uterine volume was 10.2mls (95% CI 8.3 - 12.2 ml). No subject experienced breakthrough bleeding within the first 8 months of treatment. Figure 6.1 shows the pattern of uterine growth compared to change in serum oestradiol concentrations.

After 8 months of treatment, no subject achieved a mature uterine length when taking 65 mm as the cut-off to represent maturity. Two (11%) achieved a uterine length > 57.6 mm, which represented the 5^{th} percentile in the reference group of females who had undergone spontaneous puberty from study 3. In study 3 the reported 5^{th} percentile for uterine volume was 28.5 mls and no one in the present study achieved this uterine volume after 8 months of treatment.

Of the uterine parameters at 8 months, regression analysis demonstrated that the transverse dimension was the most informative as it was most closely associated with serum oestradiol ($r = 0.80$ $p = <0.001$), compared to uterine volume ($r = 0.57$ $p = 0.03$), uterine length ($r = 0.50$ $p = 0.06$) and AP fundal measurement ($r = 0.163$ $p = 0.57$).

Breast development - Tanner staging

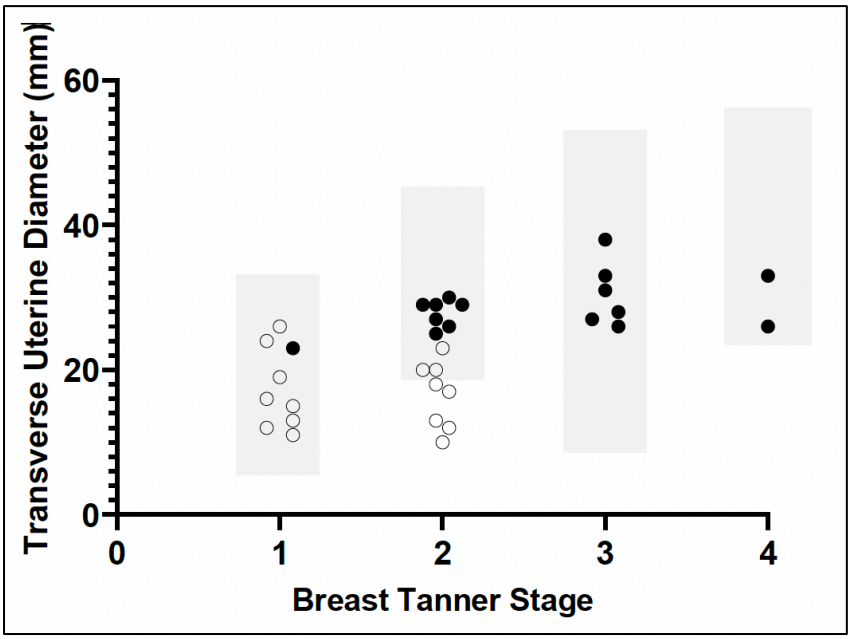
Prior to treatment all subjects had Tanner breast stage 1 or 2 (B1 50% and B2 50%). After 8 months of oestrogen treatment 7/16 (43.8%) had achieved breast Tanner stage 2 from stage 1. Six (37.5%) progressed from Tanner stages 2 to 3, and two (12.5%) progressed from Tanner stages 2 to 4. One subject (6.2%) did not progress from Tanner stage 1 and this was the same patient who did not have a change in serum oestradiol levels.

Breast Tanner stage at 8 months was weakly correlated to oestradiol levels at 8 months ($r = 0.64$ $p = 0.01$) but did not achieve significance with respect to transverse uterine measurement ($r = 0.42$ $p = 0.09$) nor uterine volume ($r = 0.48$ $p = 0.05$).

To compare this protocol for artificial puberty to reference data from normal puberty uterine transverse diameter for each Tanner stage was plotted as shown in Figure 6.3. The mean transverse diameter at each Tanner stage was 1 = 17.6mm (95% CI 13.3 - 21.9mm), 2 = 21.8mm (95% CI 18.1 - 25.6mm), 3 = 30.5mm (95% CI 25.7 - 35.2mm) and 4 = 29.5mm (95% CI - 14.9 - 73.9mm) with good approximation to the reference ranges.

Figure 6.3. Graph demonstrating the transverse uterine diameter of participants at baseline and 8 months at each breast Tanner stage compared to normative data

The open circles represent baseline data and closed circles represent 8 month data. The grey boxes represent the normative data (range) of transverse uterine dimensions adapted from Hagen et al. (46).



Breast development - 3D image assessment

Acquisition of breast images using 3D system was completed in 13/16 females. The intraobserver reproducibility for differential breast volume was assessed by intraclass correlation analysis of 0.98 (95% CI 0.97 - 0.99). Bland Altman analysis showed a mean difference between measurement of 0.63 mls with limits of agreements of - 5.48 - 6.74 mls.

Over 8 months of treatment the median total breast volume growth was 9.52 mls (5^{th} and 95^{th} percentile 1.75 - 101.2 mls). There was symmetrical increase in breast volume over 8 months of treatment with no statistical difference between the left and right volume changes ($p = 0.69$).

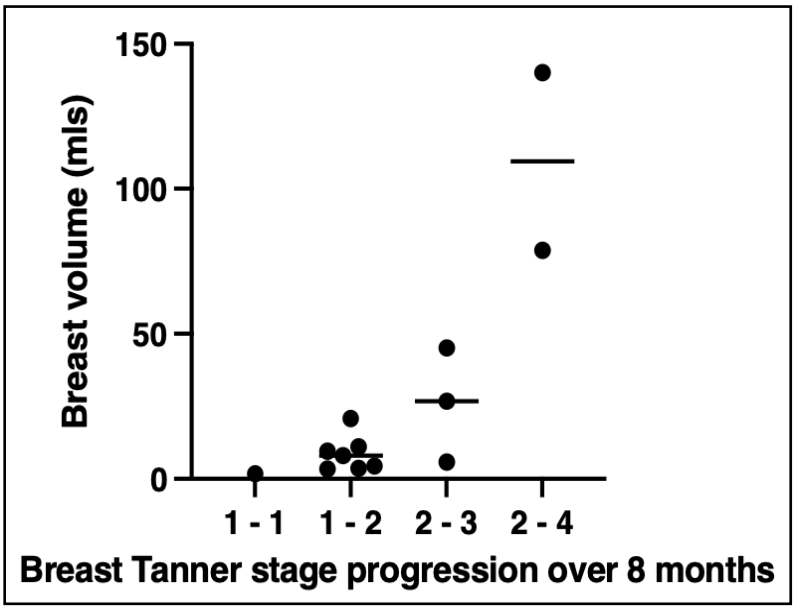
Association between Tanner breast staging and breast volume assessment

In those females who underwent breast volume assessment, 10/13 progressed one Tanner stage during the study period (1-2 or 2-3 staging), 2 females progressed 2 stages (2-4 staging) and 1 did not progress from their baseline staging. The change in Tanner stage correlated to the change in volume on 3D assessment r = 0.73 (p = 0.004).

There was a significant difference in breast volume change between the Tanner staging groups (p = 0.04). The median volume for those progressing from staging 1-2 was 8.0 mls (5^{th} and 95^{th} percentiles 3.46 - 17.0 mls), from staging 2-3 was 26.8mls (5^{th} and 95^{th} percentiles 5.75 - 32.0 mls), staging 2-4 was 109.4 mls (5^{th} and 95^{th} percentiles 78.7 - 130.0 mls). The volume change for the female that did not progress from Tanner stage 1 was 1.76mls (Figure 6.4).

Figure 6.4. Graph demonstrating the change in breast volume of each subject within each breast Tanner stage group

Line represents median breast volume change of each group.



Determinants of uterine and breast development at 8 months

Regression analysis demonstrated that the transverse diameter associated with serum oestradiol (beta standardised coefficient 0.80 p = 0.001) with no other parameter achieving significance.

Breast volume at 8 months was associated with start age (beta standardised coefficient 0.55 p = 0.04). It was not associated with uterine transverse diameter, serum oestradiol, BMI or height Z score at 8 months.

Serum oestradiol at 8 months was not associated with start age, breast volume, height Z score or BMI at 8 months.

Oestrogen dosing between 8-12 months

After completing 8 months of the defined dose treatment protocol the dose of oestradiol was individualised according to clinician assessment. Information was available for 15/16 (94%) of whom 7 (46.7%) used 37.5mcg Evorel patch and 8 (53.3%) used 50mcg Evorel patch.

The anthropometric, hormonal, bone density, uterine and breast data at 8 months for the 2 different oestradiol dosing groups is shown in Table 6.4 and Table 6.5. The biggest determinant affecting choice of dose at 8 months appeared to be the age of the patient, with those younger changing to 37.5mcg E2, however this was not statistically different. Choice of dose was not affected by oestradiol concentration, uterine assessment nor breast staging at 8 months. Whilst the FSH was statistically different between the two oestrogen dosing groups, with those having higher FSH at 8 months escalated to 50mcg, the numbers in each group were small, 4 and 3 respectively.

Table 6.4. Anthropometric, breast and hormonal at 8 months for the 2 different oestradiol dosing groups

Results are displayed as mean and 95% CI. Age is displayed in median and 5th and 95th centile. Tanner breast stage at 8 months is displayed as percentage. P values are displayed for the differences between the two oestradiol dose groups.

	Oestradiol dosing group		
	37.5mcg	**50mcg**	**P value**
Age at initiation of treatment	16 (13 -16.3)	17 (14 - 17.5)	0.28
Tanner breast stage n (%)	B2 57.1% (n=4) B3 42.9% (n=3)	B1 12.5% (n=1) B2 37.5% (n=3) B3 37.5% (n=3) B4 12.5% (n=1)	0.66
Weight (kg)	55.1 (45.5 - 64.8)	61.8 (45.2 - 78.3)	0.44
Height (cm)	161.1 (154.7 - 167.5)	163.6 (153.7 - 173.4)	0.63
Height z score	-0.24 (-1.1 - 0.6)	0.09 (-1.14 - 1.59)	0.66
BMI (kg/m2)	21.2 (17.7 - 24.6)	22.6 (18.4 - 26.8)	0.54
Hip circumference (cm)	92.2 (83.6 - 100.9)	94.6 (82.7 - 106.4)	0.71
Waist circumference (cm)	75.2 (68.3 - 82.2)	77.5 (68.5 - 86.4)	0.65
Hip waist ratio	0.81 (0.76 - 0.87)	0.82 (0.78 - 0.85)	0.90
Body fat (%)	28.8 (22.2 - 35.5)	29.9 (18 - 41.7)	0.85
LH (IU/L)	16.7 (-19.8 - 53.2)	30.2 (18.1 - 42.2)	0.61
FSH (IU/L)	23.9 (0.2 - 47.7)	70.4 (56.6 - 84.3)	0.001
sE2 (pmol/L)	88.0 (28.4 - 147.5)	61.1 (33.5 - 88.7)	0.29

Table 6.5. Uterine and bone density data at 8 months for the 2 different oestradiol dosing groups

Results are displayed as mean and 95% CI. Age is displayed in median and 5th and 95th centile. Tanner breast stage at 8 months is displayed as percentage. P values are displayed for the differences between the two oestradiol dose groups.

Oestradiol dosing group			
	37.5mcg	**50mcg**	**P value**
Total uterine length (mm)	49.4 (44.4 - 54.4)	51.0 (45.7 - 56.2)	0.61
AP Uterine Body (mm)	16.4 (12 - 20.7)	16.1 (13 - 19.2)	0.89
AP Cervix (mm)	12.7 (8.8 - 16.5)	11.8 (9.9 - 13.8)	0.65
Fundus AP: Cervix AP ratio (FCR)	1.3 (1.1 - 1.5)	1.4 (1.0 - 1.7)	0.70
Transverse (mm)	28.7 (24.6 - 32.7)	28.2 (25.6 - 30.8)	0.81
Uterine volume (mls)	11.8 (8.3 - 15.2)	12.6 (8.6 - 16.5)	0.72
Total Hip Z score	-2.1(-2.6 -1.6)	-1.3 (-2.7 - 0)	0.11
Total Hip BMC (g)	24 (21.1 - 26.9)	28.6 (18.6 - 38.6)	0.30
Total Hip BMD (g/cm^2)	0.6 (0.6 - 0.7)	0.7 (0.6 - 0.9)	0.13
Lumbar spine Z score	-2.8 (-3.5 - 2.0)	-3.4 (-5.0 - 1.9)	0.13
Lumbar spine BMC (g)	25.9 (30.5 - 41.3)	36.2 (26.7 - 45.7)	0.89
Lumbar spine BMD (g/cm^2)	0.7 (0.6 - 0.7)	0.7 (0.5 - 0.8)	0.34

The mean change in measurements between 8 and 12 months of treatment was not affected by the dose (Table 6.6).

Table 6.6. Differences in anthropometric, hormonal and uterine parameters between 8 and 12 months of treatment in the 2 oestrogen dosing groups

Results are displayed as mean and 95% CI. P values are displayed for the differences between the two oestradiol dose groups.

Oestradiol dosing group			
	37.5mcg	**50mcg**	**P value**
Weight difference (kg)	2.0 (0.2 - 3.8)	0.1 (-3.4 - 3.6)	0.28
Height difference (cm)	0.7 (0 - 1.4)	0.3 (-1.1 -1.8)	0.62
BMI difference (kg/m2)	0.5 (0 - 1.2)	0 (-1.3 -1.3)	0.39
Hip circumference difference (cm)	- 1.5 (-4.8 - 1.7)	-1.0 (-4.5 - 2.5)	0.78
Waist circumference difference (cm)	2.7 (-2.7 - 8.1)	- 2.6 (-6.8 - 1.5)	0.08
Body fat difference (%)	1.7 (0.2 - 3.2)	0.4 (-1.9 - 2.8)	0.27
FSH difference (IU/L)	7.2 (-61.0 - 75.0)	- 29.7 (-96.8 - 37.4)	0.17
LH difference (IU/L)	2.9 (-78.0 - 83.9)	-8.6 (-52.9 - 35.6)	0.61
sE2 difference (pmol/L)	80.8 (-17.3 - 178.9)	59.0 (-3.8 - 121.8)	0.65
Total Uterine length difference (mm)	6.4 (2.6 - 10.2)	9.1 (6.5 - 11.7)	0.18
AP uterine body difference (mm)	2.1 (-2.1 - 6.3)	4.8 (1.6 - 8.0)	0.24
AP cervix difference (mm)	2.4 (-1.4 - 6.2)	2.6 (0.8 - 4.5)	0.89
Fundus AP: Cervix AP ratio difference	0.0 (-0.3 - 0.2)		
Transverse difference (mm)	4.8 (0.1 - 9.5)	6.5 (5.0 - 7.9)	0.43
Uterine volume difference (ml)	6.8 (3.9 - 9.8)	9.9 (4.8 - 15.0)	0.19

After 12 months of treatment 6/13 (46.1%) achieved a uterine length above the 5th centile of the normal range but only 2/13 (15.3%) achieved a uterine volume above the 5th centile of the normal range respectively. Between 8 and 12 months 3 females experienced break through bleeding (2 taking 50mcg and 1 taking

37.5mcg patch dose). By 12 months of treatment 66.7% (n=10) and 26.7% (n=4) reached Tanner breast stage 3 or 4 respectively. One female progressed from Tanner stage 1 to Tanner stage 2 between 8 to 12 months of treatment.

Summary

In summary, after 8 months of treatment, the changes in oestradiol concentrations (0 - 174 pmol), uterine volume growth (4.4 – 16.4mls) and breast volume (1.76 - 140.1mls) varied greatly between individuals. Of uterine parameters, transverse uterine diameter was most closely associated with serum oestradiol levels at 8 months (beta standardised coefficient 0.80 $p = 0.001$). Change in breast volume was associated with age of treatment initiation (beta standardised coefficient 0.55 $p = 0.04$).

CHAPTER 7

STUDY 5 RESULTS

QUANTITATIVE ASSESSMENT OF BREAST VOLUME USING 3D IMAGING IN TRANS FEMALES: A PILOT STUDY

I wished to explore the application of 3D breast imaging in the trans female cohort to provide a quantitative assessment of breast development and volume change with oestrogen treatment.

Four participants undergoing induced breast development with exogenous oestradiol were recruited. The participant age, anthropometric measurements, oestradiol concentration and breast assessments at baseline and after 6 months of treatment can be seen in Table 7.1.

Table 7.1. Table demonstrating the age, anthropometric, hormonal and breast volume of participants at baseline and after 6 months of treatment

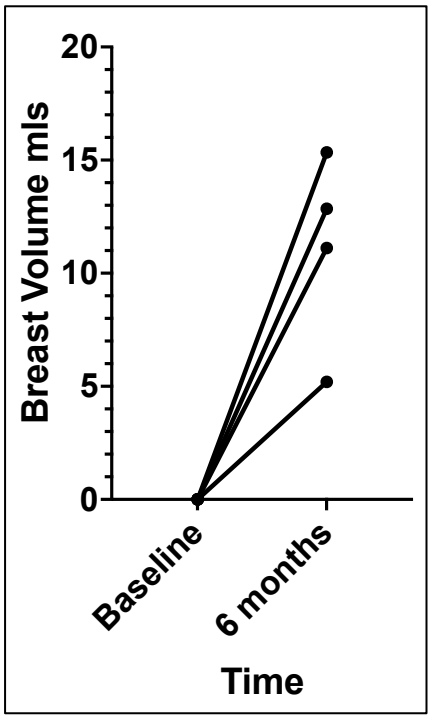
Participant	Age at oestrogen treatment initiation (years)	Exogenous treatment	Height Baseline - 6 months (m)	BMI Baseline - 6 months (kg/m2)	Oestradiol Baseline (pmol/L)	Oestradiol 6 months (pmol/L)	Total breast volume (mls)
1	15	Transdermal 12.5mcg	168 - 170	31.3 - 31.7	<44	<44	15.34
2	16	Oestradiol valerate 2mg	186 - 190	22.3 - 23.7	<44	125	5.20
3	17	Oestradiol valerate 1mg	165 - 170	17.5 - 17.0	<44	165	12.85
4	17	Oestradiol valerate 2mg	185 - 186	23.8 - 21.7	<44	120	11.11

All participants had been taking GnRHa for at least one year and had undetectable serum oestradiol and testosterone concentrations prior to the

initiation of oestradiol therapy. Out of the 4 participants; two started on oestradiol valerate 2mg taken once daily orally, 1 started oestradiol valerate 1mg taken once daily orally and 1 started transdermal oestradiol patch 12.5mcg (patch changed twice per week).

After 6 months of treatment, the oestradiol concentration ranged between <44 – 165 pmol/L. Differential breast volume after 6 months of oestradiol treatment ranged between 5.2mls and 15.3mls (Figure 7.1). Volume change was detected even in the participant in whom the serum oestradiol concentration remained below the level of detection. Visual inspection demonstrated maximal growth in the areola and nipple area. All participants started with Tanner stage 1 and all developed Tanner stage 2 after 6 months of treatment with development of breast budding.

Figure 7.1. Graph demonstrating absolute change in breast volume for each subject between treatment initiation and 6 months



Summary

In summary, in a small group of 4 individuals, following 6 months of oestradiol treatment the differential breast volume ranged between 5.2mls and 15.3mls. There was an increase in breast volume in one individual without concomitant changes in E2 levels, suggesting that breast imaging may be more informative than blood tests especially in the absence of sensitive oestradiol assays in the early stages of treatment.

CHAPTER 8

STUDY 6 RESULTS

FERTILITY AND PREGNANCY OUTCOMES IN WOMEN WITH TURNER SYNDROME AND PREMATURE OVARIAN INSUFFICIENCY

I wished to examine the fertility and pregnancy outcomes in women with Turner syndrome (TS) and premature ovarian insufficiency (POI) undergoing oocyte donation treatment or spontaneous pregnancy. General population reference data were used as comparators.

Participants with Turner syndrome

Seventy - four women underwent OD treatment and 31 women had spontaneous conception. Three women had both OD treatment and spontaneous pregnancies. The clinical characteristics of each group are shown in Table 8.1.

Table 8.1. Participant characteristics and pre pregnancy uterine dimensions in women with TS undergoing either OD treatment or with spontaneous pregnancy

Results displayed as either median & 5th - 95th centile or percentage. Note 3 women had both an OD treatment and a spontaneous pregnancy. P values displayed for the subgroup analysis between the two groups.

Turner Syndrome			
	TS OD treatment	**TS spontaneous**	**P value**
Number of women	**74**	**31**	
Height (m)	1.50 (1.39 - 1.59)	1.50 (1.42 - 1.71)	0.53
BMI (kg/m^2)	25.6 (20.0 - 36.6)	24.5 (20.3 - 37.5)	0.46
Monosomy X	28/67 (41.8%)	1/30 (3.3%)	<0.001
Cardiac pathology	16/74 (21.6%)	4/31 (12.9%)	0.23
Hypothyroidism	23/74 (31.1%)	4/31 (12.9%)	0.05
Primary amenorrhoea	60/74 (81.1%)	2/31 (6.5%)	<0.001
Uterine length (mm)	64 (37.4 - 81.8)	69 (57.0 - 80.0)	0.36
Uterine anterior posterior (mm)	28.5 (13.4 - 36.8)	30 (20.0 - 45.1)	0.70
Transverse (mm)	34.5 (26.1 - 51.0)	48.0 (40.0 - 52.0)	0.006
Uterine volume (ml)	31.7 (9.0 - 67.9)	50.5 (29.2 - 80.0)	0.04

Karyotype analysis was recorded in 93 (91.1%) women with TS. In those undergoing OD treatment, 28 had monosomy X, 17 isochromosome X, 9 mosaic 45, X/46, XX, 6 mosaic 45, X/46, XY, 6 mosaic 45, X/46, XrX and 1 partial X deletion. Sixteen (21.6%) women had a cardiac pathology: 14 bicuspid aortic valve and 2 previous surgery for aortic coarctation. Eight (10.8%) women had pre-existing HTN and 5 (6.8%) pre-existing diabetes. Twenty-three (31.1%) women had treated hypothyroidism. Sixty (81.1%) had primary amenorrhoea, 13 (17.6%) had secondary amenorrhoea and 1 (1.4%) had a regular cycle.

Karyotypes in those with TS and spontaneous conception were 14 with mosaic 45, X/46, XX, 6 complex anomalies, 4 partial X deletion, 2 mosaic 45, X/46, XY, 2 mosaic 45, X/46, XrX, 1 isochromosome X and 1 monosomy X. Four (12.9%) had a cardiac pathology: 2 bicuspid aortic valve, 1 previous surgery for aortic coarctation and 1 valvular surgery. No women had existing HTN or diabetes. Four (12.9%) women had treated hypothyroidism. Two (6.5%) had primary amenorrhoea, 3 (9.7%) had secondary amenorrhoea and 26 (83.9%) had a regular menstrual cycle.

Women with TS who had a spontaneous pregnancy were younger than those undergoing OD pregnancy. There was no difference in height or BMI between the two conception modes however those undergoing OD were more likely to have 45, X, and primary amenorrhoea compared to those with spontaneous pregnancies.

Ninety-one (89.2%) women had an echocardiogram prior to pregnancy and 49 (48%) had a dedicated pre pregnancy cardiology consultation. Aortic size index (ASI) results were available for 81 women (79.4%) as 8 women had echocardiogram examination in their local hospital and two women had a cardiology assessment with echocardiogram and the aortic measurements were described as normal but no ASI was reported. ASI was greater than 2 cm/m^2 in 16/81 (19.7%) and 12 of these women had a specialist cardiology review also. No woman had an ASI >2.5 cm/m^2.

Participants with POI

Fifty-three women with POI underwent OD treatment and 18 had spontaneous conception. Three women had both OD treatment and spontaneous pregnancies.

The clinical characteritics of each group are shown in Table 8.2. Six women had a previous pregnancy prior to the diagnosis of POI.

Table 8.2. Participant characteristics in women with POI undergoing either OD treatment or with spontaneous pregnancy

Results displayed as either median & 5th - 95th centile or percentage. P values displayed for the subgroup analysis between the two groups.

Premature Ovarian Insufficiency			
	POI OD treatment	**POI spontaneous**	**P value**
Number of women	53	18	
Age at diagnosis	25.0 (14.7- 39.0)	25.0 (15.0 - 36.0)	0.28
Height (m)	1.65 (1.51 - 1.77)	1.65 (1.51 - 1.72)	0.90
BMI (kg/m^2)	22.7 (18.9 - 34.9)	23.3 (19.0 - 27.9)	0.67
Hypothyroidism	11/53 (20.8%)	1/18 (5.6%)	0.13
Primary amenorrhoea	11/53 (20.8%)	1/18 (5.6%)	0.13
Uterine length (mm)	64.0 (44.0 - 74.1)	65.0 (56.0 - 70.1)	0.90
Uterine anterior posterior (mm)	24.0 (19.0 - 39.2)	29.0 (19.0 - 31.0)	0.73
Transverse (mm)	35.0 (28.0 - 43.0)	33.0 (29.0 - 44.0)	0.92
Uterine volume (ml)	28.7 (14.3 - 54.0)	33.4 (17.6 - 50.2)	0.72

Fragile X premutation had been identified in 5 women (4 undergoing OD treatment and 1 with spontaneous conception). In those undergoing OD treatment 11 (20.8%) had treated hypothyroidism and 11 (20.8%) had a history of primary amenorrhoea. No women had pre-existing HTN or diabetes. In those with spontaneous conception 1 (5.6%) had treated hypothyroidism, 1 (5.6%) had a history of primary amenorrhoea and 1 (5.6%) had pre-existing diabetes. The median duration between POI diagnosis and first subsequent spontaneous pregnancy was 2 years.

In those with POI there was no difference in age of diagnosis, height, BMI or the prevalence of treated hypothyroidism or primary amenorrhoea between the two conception modes. The regression model did not identify any predictive factors for spontaneous conception.

Oocyte donation fertility treatment

Information regarding participants undergoing OD and outcomes can be seen in Table 8.3. Seventy-four women with TS and 53 women with POI underwent OD. Seven women (3 TS and 4 POI) had a spontaneous pregnancy prior to embarking on fertility treatment. In those undergoing OD, women with TS were significantly shorter and had a higher BMI compared to those with POI. The prevalence of treated hypothyroidism was not different between TS and POI. Women with TS were significantly more likely to have primary amenorrhoea than those with POI.

Information was collected for 332 cycles; 196 cycles in women with TS and 136 in women with POI. Nine cycles in 7 different women did not result in embryo transfer and were abandoned (4 with TS and 5 with POI). The median number of cycles in women with TS was 2 (5^{th} - 95^{th} percentile 1-7) and in those with POI was 2 (5^{th} - 95^{th} percentile 1-5). There was no difference in the age of first treatment cycle between TS and POI.

Table 8.3. Fertility treatment outcomes in women with Turner syndrome or Premature Ovarian Insufficiency undergoing oocyte donation treatment.

Results displayed as either median & 5th - 95th centile or percentage. P values displayed for the subgroup analysis across the different diagnostic groups

	Oocyte Donation Subgroup		
	TS	**POI**	**P Value**
Total number of women	**74**	**53**	
Height (m)	1.50 (1.39 - 1.59)	1.65 (1.51 - 1.77)	<0.001
BMI (kg/m^2)	25.6 (20.0 - 36.6)	22.7 (18.9 - 34.9)	0.008
Hypothyroidism	23/74 (31.1%)	11/53 (20.8%)	0.19
Primary amenorrhoea	60/74 (81.1%)	11/53 (20.8%)	<0.001
Number of cycles	196	136	
Number of women with prior pregnancy	3	4	
Number of cycles per woman	2 (1 - 7)	2 (1 - 5)	0.63
Age at first treatment (years)	33.0 (25 - 43.5)	33.0 (25.7 - 43.3)	0.78
Abandoned cycles	4/196 (2%)	5/136 (3.7%)	0.36
Uterine length (mm)	64.0 (37.4 - 81.8)	64.0 (44.0 - 74.1)	0.46
Uterine anterior posterior (mm)	28.5 (13.4 - 36.8)	24.0 (19.0 - 39.2)	0.36
Transverse (mm)	34.5 (26.1 - 51.0)	35 (28.0 - 43)	0.93
Uterine volume (ml)	31.7 (9.0 - 67.9)	28.7 (14.3 - 54)	0.68
Pregnancy rate per cycle started	105/196 (53.6%)	84/136 (61.8%)	0.13
Live birth rate per cycle started	61/196 (31.1%)	62/136 (45.6%)	0.007

Comparison of fertility outcomes between TS and POI

In women with TS, the clinical pregnancy rate pregnancy rate and live birth rate per cycle started was 53.6% and 31.1% respectively. Whilst the pregnancy rate per cycle started was not different between the those with POI or TS, the live birth rate per cycle started was lower in those with TS than POI (TS 31.1% vs POI 45.6% p = 0.007). Regression analysis demonstrated that when adjusted for age and cycle number, TS was associated with lower live birth rate per cycle started (OR 0.53 95% CI 0.34 - 0.84 p = 0.008) with no other parameter reaching significance.

Pregnancy Outcomes in TS after OD

Information regarding pregnancy outcomes can be seen in Table 8.4. Sixty-five women with TS had a total of 105 pregnancies after OD. Sixty-one pregnancies (58.1%) resulted in live birth and 70 children were born; 53 singletons, 7 sets of twins and 1 set of triplets. Forty-two (40%) pregnancies ended in miscarriage and 2 (1.9%) resulted in ectopic pregnancy.

There was no case of aortic dissection during pregnancy in those with TS and OD pregnancy. In those with TS and OD singleton pregnancies, excluding those with pre-existing HTN or diabetes, the incidence of HTN pathology was 8/48 (16.7%) and GDM 4/51 (7.8%). Caesarean section was the mode of delivery in 44/53 (83%) (emergency LSCS in 17/44 38.6% and elective LSCS in 27/44 61.4%). The incidence of preterm birth and SGA was 17.6% and 35.4% respectively.

Table 8.4. Pregnancy outcomes in women with TS undergoing either OD or spontaneous pregnancy compared to women with POI and OD pregnancy and general population data. Pregnancy data for women with POI and OD pregnancy compared to spontaneous pregnancy and general population data

Results displayed as either median & 5th - 95th centile or percentage. P values displayed for the subgroup analysis of modes of conception in those with TS, between diagnostic groups or compared to general population data. a - significantly different than general population data (all pregnancies), b - significantly different from general population data (spontaneous singleton pregnancies).

	TS OD	TS spont	TS OD vs TS Spont p value	POI OD	POI Spont	POI OD vs POI Spont p value	TS OD vs POI OD p value	Normative data Spontaneous singleton pregnancy
Women (n)	**65**	31		**50**	18			
Pregnancies (n)	105	71		84	28			
Number of children born	70	45		73	20			
Live birth rate	61/105 (58.1%)	43/71 (60.6%)	0.74	62/84 (73.8%)	19/28 (67.9%)	0.54	0.02	
Miscarriage rate	42/105 (40%)	23/71 (32.4%) [a]	0.30	22/84 (26.2%)	7/28 (25%)	0.9	0.04	15.3%
Termination of Pregnancy	0	5/71 (7%)		0	1/28 (3.6%)			
Intrauterine Death	0	0		0	1/28 (3.6%)			
Singletons	53/61 (86.9%)	41/43 (95.3%)		51/62 (82.3%)	18/19 (94.7%)			

	TS OD	TS spont	TS OD vs TS Spont p value	POI OD	POI Spont	POI OD vs POI Spont p value	TS OD vs POI OD p value	Normative data Spontaneous singleton pregnancy
Twins (sets)	7/61 (11.5%)	2/43 (4.7%)		11/ 62 (17.7%)	1/19 (5.3%)			
Triplets (sets)	1/61 (1.6%)	0		0	0			
Age at pregnancy	34 (26 - 43.4)	27 (18 - 39)	<0.001	34.5 (27 - 43.5)	31 (20.9 - 41)	<0.001	0.14	
HTN pathology	8/48 (16.7%)	5/40 (12.5%) [b]	0.56	10/48 (20.8%)	2/17 (11.8%) [b]	0.40	0.60	2.8%
GDM	4/51 (7.8%)	6/40 (15%) [b]	0.27	8/48 (16.7%)	2/15 (13.3%) [b]	0.75	0.17	3.7%
LSCS	44/53 (83%)	21/39 (51.2%) [b]	0.005	28/49 (57.1%)	5/17 (29.4%)	0.05	0.004	16.9%
Preterm birth	9/51 (17.6%)	2/38 (5.3%)	0.07	4/47 (8.5%)	2/14 (14.3%)	0.52	0.18	4.7%
SGA	17/48 (35.4%)	6/38 (15.8%) [b]	0.04	13/47 (27.7%)	2/13 (15.4%) [b]	0.36	0.41	2.8%

Pregnancy outcomes in TS spontaneous pregnancy

Thirty-one women with TS had 71 spontaneous conceptions. Pregnancy outcome data can be seen in Table 8.4. Forty- three pregnancies (60.6%) resulted in live birth and 45 children were born; 41 singletons and 2 sets of twins. Twenty - three pregnancies (32.4%) resulted in miscarriage. Five termination of pregnancies (TOPs) were recorded in 4 women, the reason being social in 3 women and non-disclosed in the 4th.

There was no case of aortic dissection. In singleton pregnancies the incidence of HTN pathology and GDM was 12.5% and 15% respectively. The mode of delivery was LSCS in 51.2% (13 elective and 8 emergency). The rate of preterm delivery was 2/38 5.3% and SGA was 6/38 15.8%.

There were 29 female (64.4%) and 16 male babies (35.6%) born to women with TS and spontaneous pregnancy, giving a M:F ratio of 0.55. which is significantly different than the expected 24:21 based on the ratio of 1.06 in the general population p = 0.01 (249).

There were 5 cases (17.2%) of daughters born with Turner syndrome. The associated maternal karyotypes were 2 with 46, X,t(X;Y)(p22.3;q11), 1 with 46X,del(X) (Xq21.3), 1 with 46,X,r(X) and 1 with 46X,del(X) (p21.1). In the first 3 cases the daughter's karyotype were the same as the mother's and in the latter cases the daughter's karyotype were unknown. Two cases of vertical transmission occurred over 3 generations within the same family.

Comparison of mode of conception in TS

Compared to TS spontaneous pregnancies, the rate of LSCS was significantly higher in those with OD pregnancy (83% vs 51.2% p = 0.005) as was the rate of

SGA (35.4% vs 15.8% p = 0.04). There was no difference in the rate of live birth, miscarriage, HTN, GDM or preterm delivery. Regression analysis adjusting for age at pregnancy and parity showed the raised rates of both LSCS (OR 4.19 95% CI 1.61 - 10.8 p = 0.003) and SGA (OR 2.92 95% CI 1.02 -8.38 p = 0.04) remain significant with no other parameter reaching significance.

TS spontaneous pregnancies compared to population reference data

The incidence of miscarriage in women with TS and spontaneous pregnancy was higher than the miscarriage rate in all recognised pregnancies in the general population of 15.3% (323) p = <0.001. Compared to population data of spontaneous singleton pregnancy, TS spontaneous singleton pregnancies were associated with an increased rate of HTN pathology (12.5% vs 2.8% p = <0.001), GDM (15% vs 3.7% p = <0.001), LSCS (51.2 % vs 16.9% p = <0.001) and SGA (15.8 % vs 2.8% P = <0.001). There was no significant difference in the rate of preterm delivery.

Pregnancy Outcomes in women POI

Information regarding pregnancy outcomes in women with POI can be seen in Table 8.4.

Fifty women with POI had 84 OD pregnancies. The pregnancy outcomes can be seen in Table 9.4. Sixty-two pregnancies (73.8%) resulted in live birth and 73 children were born; 51 singletons and 11 sets of twins. Twenty-two (26.2%) pregnancies ended in miscarriage. In those with OD singleton pregnancies the incidence of HTN pathology was 20.8% and GDM 16.7%. Caesarean section was the mode of delivery in 28/49 (57.1%) (emergency LSCS in 9/28 32.1% and

elective LSCS in 19/28 67.9%). The incidence of preterm birth and SGA was 8.5% and 27.7% respectively.

Eighteen women with POI had 28 spontaneous pregnancies. Nineteen pregnancies (67.9%) resulted in live birth and 20 children were born; 18 singletons and 1 set of twins. Seven (25%) pregnancies ended in miscarriage, 1 (3.6%) ended in termination of pregnancy and 1 (3.6%) resulted in IUD. In those with spontaneous singleton pregnancies (both live birth and IUD) the incidence of HTN pathology was 11.8% and GDM 13.3%. Caesarean section was the mode of delivery in 5/17 (29.4%) emergency LSCS 3/5 60% and elective in 2/5 40%). The incidence of preterm birth and SGA was 14.3% and 15.4% respectively.

There was no difference in the rate of live birth, miscarriage, HTN, GDM, LSCS, SGA or preterm delivery between the modes of conception in females with POI.

Comparison of OD pregnancy outcomes between TS and POI

The pregnancy outcomes can be seen in Table 8.4. In the OD groups, those with TS compared to POI had lower live birth and increased miscarriage rate (live birth 58.1% vs 73.8% p = 0.02 and miscarriage 40% vs 26.2% p = 0.04) which both remained significant with regression analysis adjusting for age and gravidity; OR for live birth of 0.49 (95% CI 0.26 -0.91 p = 0.02) and an OR for miscarriage of 1.87 (95% CI 1.00 – 3.50 p = 0.04) with no other factor reaching significance. The rate of LSCS was greater in those with TS pregnancy (83% vs 57.1% p = 0.004) and when adjusted for parity and age at pregnancy the OR was 4.26 (95% CI 1.63 – 11.1 p = 0.003). No difference between the diagnostic groups was identified in the rate of HTN pathology, GDM, preterm birth or SGA.

POI spontaneous pregnancies compared to population reference data

In women with POI and spontaneous pregnancy, there was no difference in the rate of miscarriage compared to population data. There were increased rates of HTN (11.8% vs 2.8% p = 0.03), GDM (13.3% vs 3.7% p = 0.03) and SGA (15.4% vs 2.8% p = 0.001) compared to population data.

Uterine Size

Fifty-five women (32.4.%) had pelvic ultrasound scans prior to fertility treatment or pregnancy from which measurements of uterine dimensions could be recorded (Table 8.1 and Table 8.2).

Thirty-two women with TS (23 OD and 9 spontaneous conception) had a pelvic ultrasound. In those with TS the uterine transverse and uterine volume measurements were significantly smaller in the OD group compared to those with spontaneous pregnancy (transverse 34.5 mm vs 48 .0 mm p = 0.006; volume 31.7mls vs 50.5mls p = 0.04).

Twenty-three women with POI (19 OD and 4 spontaneous conception) had a pelvic ultrasound, there was no difference in uterine measurements between the conception groups.

Summary

In summary, in those with TS, OD pregnancies were associated with increased rates of LSCS and SGA compared to spontaneous pregnancies; LSCS (OR 4.19 95% CI 1.61 – 10.8 p = 0.003) and SGA (OR 2.92 95% CI 1.02 -8.38 p = 0.04). There were no recorded cardiac events but 5 (17.2%) cases of vertical transmissions of TS in daughters were identified. Oocyte donation in those with

TS was associated with lower live birth rate per cycle started (OR 0.53 95% CI 0.34 - 0.84 p = 0.008) and a higher rate of miscarriage compared to women with POI (40% vs 26.2% p = 0.04). Compared to population data in women with TS and spontaneous conception the rate miscarriage was higher as was the rate of HTN pathology (12.5% vs 2.9% p = <0.001), GDM (15% vs 3.7% p = <0.001), LSCS (51.2 % vs 16.9% p = <0.001) and SGA (15.8 % vs 2.7% P = <0.001).

In those with POI and spontaneous conceptions the miscarriage rate was not higher than reference data however there were increased rates of HTN, GDM and SGA.

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CHAPTER 9

STUDY DISCUSSIONS

STUDY 1 DISCUSSION - EXAMINATION OF THE REPRODUCIBILITY OF UTERINE DIMENSIONS USING TRANSABDOMINAL ULTRASONOGRAPHY COMPARED TO TRANSVAGINAL ULTRASONOGRAPHY AND MRI

This study has demonstrated that excellent inter and intraobserver reproducibility can be achieved for TAUS determination of uterine size when following a standardised protocol, providing reassurance for its use to provide serial measurements. Interchanging between modalities in the same individual however to provide longitudinal assessment should be used with caution due to the lower level of ICC agreement.

Different measurements have been utilised in the assessment and definition of uterine maturity in the context of pubertal induction (116, 124, 143, 147, 150, 153). Historically uterine length has been commonly used, with 65mm thought to represent maturity (71, 124, 143, 144) but the transverse measurement and uterine volume may be more appropriate with superior association to oestradiol levels compared to uterine length, as demonstrated in study 4. Furthermore, the total uterine length should be used clinically due to the limitations and difficulty in assessing the correct anatomical landmarks which demarcate the junction between uterine body and cervix on transabdominal scan (16). I demonstrated that the interobserver variability between the uterine body length and cervix length varied but ultimately did not affect the total uterine length. Overall, the inter and intra observer reproducibility of TA approach was excellent in my study.

For females who are virgo intacta, a transabdominal approach is the standard methodology of assessing uterine size, however once sexually active then a

vaginal approach may be considered. Whilst it has been reported that TA and TV measurements may be comparable for the detection of some pelvic pathology (16, 18, 67, 331, 332), data examining the agreement between TAUS and TVUS of uterine size and volume is lacking and not conclusive. It has been suggested that, using cohorts of scans, generally smaller uterine sizes are obtained using TVUS (16), whereas others reported no difference (18). I found a discrepancy in the measurement of uterine body and cervix length between the TA or TV approach. The uterine body length appeared consistently greater and cervix length less when measured with TAUS compared with TVUS. The uterine body AP and cervix AP measurements were also different. The uterine body AP measurements were consistently smaller with the TAUS and cervix AP measurements smaller with TVUS, which may be secondary to bladder or probe compression respectively. Whilst there was no difference in uterine volume between the two approaches, there were consistently poorer ICC levels between the two approaches with wide 95% CI, suggesting that for completion of longitudinal assessment a single approach for the entirety should be advocated.

There is scant and conflicting data comparing uterine size between MRI and US (46, 77, 80, 333). Hagen et al. examined uterine volume in females who underwent both TAUS and MRI (46). They reported a difference in the uterine measurements, with total uterine length and width, and therefore volume, being greater with TAUS compared to MRI (46). On the contrary Cleemann et al. examined uterine size in females with Turner syndrome and whilst they found comparable measurements of uterine volume between US and MRI in those with Tanner staging 1-4, in those with Tanner stage 5, MRI produced larger uterine volumes estimates than TAUS (80). This study demonstrated that whilst the mean difference in the uterine parameters was not significantly different between

TAUS and MRI measurement there was poor ICC between the two modalities. MRI is limited as a routine everyday clinical tool due to time, availability and financial constraints however it may prove beneficial in those where an optimal TA scan is challenging or when other pathology is suspected.

The strength of the study is a robust study design. I endeavoured to ensure accuracy by using real time examinations rather than static images from one acquisition, blinding of the second observer and using direct comparison of TAUS and TVUS / MRI in the same patient rather than relying on cohorts of scans (334, 335).

In conclusion TAUS should remain the gold standard for the evaluation of uterine size in adolescents and young adults and should be performed by experienced practitioners following a standard protocol.

STUDY 2 DISCUSSION - CHALLENGES IN DEVELOPING A QUANTITATIVE METHOD OF MEASURING BREAST DEVELOPMENT USING 3D IMAGING - AN EXAMPLE OF A NOVEL METHOD FOR USE IN INDUCED BREAST DEVELOPMENT WITH EXOGENOUS OESTROGEN

Optimal breast development is essential in the context of exogenous oestrogen treatment and I have demonstrated a promising novel application of 3D breast imaging providing longitudinal breast volume assessment in those undergoing pubertal induction treatment. I have demonstrated that 3D breast differential volume assessment, without the need for landmarking and presence of mature breast tissue, can be achieved with good validity and reproducibility.

Alignment of 2 breast images taken at different time points during treatment allows breast volume change to be calculated without the need for landmarking. Current application of 3D breast imaging in the field of adult breast surgery relies on landmarking to define the contour of the breast. The landmarks can either be placed manually or automated by the software (200, 209). Even with mature breast tissue there are several inherent problems with landmarking and the detection of breast borders, which in turn affects the accuracy of the digital interpolation of the posterior plane and ultimately the volume calculated (200, 202, 213).

Prior to oestradiol therapy there is minimal breast tissue and the automated systems for mapping the extremities of the breast tissue would be anticipated to have poor accuracy. During the protocol planning stage, several different methods for manual landmarking on the patient or on the captured image were tested. Landmarking was deemed near impossible as there were often no borders to define. As a result, reproducibility was the biggest barrier. Other methods have been proposed to aid delineation of the breast border including the folding line method (336) but again, relies on mature breast tissue. Furthermore, the need for direct manual landmarking may not be acceptable in these younger patients due to the time of exposure required.

I found that landmarking was compromised and inaccurate even when more mature breast tissue was present. Other authors have also highlighted the challenges faced with breast anatomical landmarking with significant variation in measurements between direct anthropometric landmarking on the individual and then marking the 3D image (200). The breast base width and nipple to inframammary fold measurements appear to be most inaccurate with mean differences of over 1cm seen between the two methods (200). Similar to my

findings, Steen et al. demonstrated that manual manipulation of the images did not circumvent this landmarking difficultly and accurate delineation of the border between the breast tissue and the chest wall is difficult to assess. Without palpating the chest wall and breast, one is relying only the change in contour on the image and shadowing, which is not sufficient (200). Accurate volume assessment with mature breast tissue has been hindered by ptosis due to underestimating the inferior surface of the breast, patient positioning, prominent pectoralis muscle and adiposity which may conceal the exact breast boundaries (204, 213).

Reproducibility of repeated imaging and measurements may be related to several factors related to both the subject and the examiner (206). Anatomy and tissue definition may vary depending on factors patient positioning including sitting angle, head and arm placement and respiratory effort (202, 206-208, 211). Overlaying two static images of the same male subject taken on different occasions using a positioning protocol with a bespoke posture support resulted in minimal calculated differential volume, demonstrating that breast volume was not a consequence of artefact or positioning. Furthermore, the intraobserver reproducibility of this process and measurements calculated was reassuring suggesting that volume change was not examiner or measurement technique dependent. Reassuringly intra and interobserver reproducibility of the volume assessment by 3D breast imaging has also been demonstrated in other applications (202, 212, 215).

3D breast volumetry calculated by software in the context of adult breast surgery has been compared to other methods including MRI, clinician estimation and excised tissue volume (193, 202, 212-214, 337) where the breast volumes range was approximately 250 - 1000 mls (338). In the early stages of oestrogen therapy,

smaller volume changes were expected, so I therefore needed to consider other methods for validating my measurements.

Computer generated displacement of a static image by a defined measurement provided a reference volume to be generated. I demonstrated good validity and no statistical difference between the reference volume and the volume calculated by image alignment. MRI was considered however shape and size of the breast may be affected by the supine positioning required for MRI (193).

I demonstrated the application of this novel technique in those undergoing oestrogen treatment with excellent reproducibility. Longitudinal volume assessment would provide more insight into the natural history of breast development and would offer more guidance on response to treatment and individualised dose adjustments.

I appreciate that this study did not attempt to assess discrimination between glandular and adipose components as might be determined using ultrasound. Ultrasound, however, requires greater length of exposure time and may therefore be less suitable for routine use.

In conclusion, this is a preliminary study examining the possibility of 3D breast volumetry in the context of small and subtle changes in breast volume. Rate of volume change is the most important aspect of early induced breast development to enable identification of those requiring accelerated increments of oestrogen dose. Tanner staging can be complemented by 3D breast assessment to optimise the outcomes of treatments.

STUDY 3 DISCUSSION - UTERINE VOLUME AFTER INDUCTION OF PUBERTY IN WOMEN WITH HYPOGONADISM

This study demonstrates that standard oestrogen regimens for induction of puberty result in reduced uterine size in women with hypogonadism compared to their peers who have undergone spontaneous puberty. I showed that serum oestradiol levels may influence the adult configuration but most striking was the wide range of uterine size achieved in this cohort with many failing to obtain uterine measurements above the 5th percentile of the reference group.

I found that 24% of women with hypogonadism had reduced uterine length and 48% reduced volume being below 5th percentile of the reference group respectively. In contrast to my findings, several papers report that with oestrogen therapy, adequate uterine size can be achieved. Using only uterine length, McDonnell et al. (148) found that all of 18 women with TS achieved a length between 50 - 80 mm which, based on normative data, was deemed mature. However, Paterson et al. (58) undertook a study of 38 women with TS who underwent pubertal induction receiving ethinylestradiol and defined uterine maturity based on both length and AP fundal cervical ratio (FCR). They concluded that of those who achieved Tanner breast stage 5, only 50% were documented to have an FCR greater than 1:1, despite a relatively normal uterine length (60mm). This highlights the importance of the appreciation of uterine size in its entirety rather than relying on a single measurement, as this may provide false reassurance in terms of adequate development. Furthermore, despite a reported increase in uterine size with therapy, there may still be a shortfall compared to those who have undergone spontaneous puberty.

In the absence of a universally agreed definition of mature uterine dimensions, and the anatomical landmarks that should be used for measurement, I chose to compare the uterine parameters to a reference group using a set protocol for measurement. There is variation in the literature as to the criteria comprising uterine maturity. For uterine length, 65mm is commonly used for maturity (124, 143, 147), which is based on the normative data by Griffin et al. (71). The ratio of fundus to cervix (FCR) is also described and some use an amalgamation of both to describe the uterus as immature, transitional or mature (143, 147, 150, 152). Until recently, reference data has been limited to small cohorts and younger ages, however age matched uterine volume data has been reported by Kelsey et al. from birth to 40 years of age (19). This model combined both MRI images and historical published ultrasound data to acquire uterine volumes. Gilligan et al. examined the retrospective ultrasound examinations of 889 patients between the ages of 0-20 years to provide nomograms for uterine parameters based on age (18). The methodology of both of these reports varies from my technique of single observer ultrasound.

It has been postulated that an optimal window for uterine development may exist in adolescence, and that outside of this, reduced size cannot be subsequently rectified and ‘catch up’ is not possible. The exact nature of this window has not been fully defined. Snajderova et al. noted a negative correlation between age of menarche and uterine length suggesting that late menarche is associated with a smaller uterus (143). Nakamura however reported that uterine length was not affected by whether treatment was started early or late although the cut-off for late puberty at 18 years in this study may have been too late to detect an effect in early adolescence (144). I found no significant correlation between age of initiation of therapy or menarche age on uterine size.

Nabhan et al. examined the use of transdermal 17β-oestradiol patch compared with conjugated equine oestrogen in girls with TS undergoing pubertal induction in a prospective randomised controlled trial (124). There was superiority of the transdermal patch in terms of bone indices, uterine growth and breast development. My study did not find any differences in the final uterine size based on the type of oestradiol preparation used for initiation of therapy, but there was a trend in those that had the COCP having smaller uterine volume measurements compared to those who started on transdermal preparations.

It has been reported that height and weight may be determinants of uterine size with shorter women having smaller uteri. This may reflect the role of other adjuvant hormones such as GH and insulin like growth factor 1 (IGF-1) acting in a synergistic fashion with oestrogen to influence uterine growth (143). Indeed the early uterine growth, occurring independently of oestrogen is hypothesised to be perhaps related to GH as the uterine size in prepubertal females was found to be correlated with height (20). Sampaola et al. found a beneficial effect of GH use on uterine volume in girls with TS as did Mondal et al. (160, 161). It may therefore be expected that a relationship between growth and uterine size may exist if the two are directly interlinked. Data on this topic from the literature are not conclusive. Using cross-sectional data, Elsedfy et al. examined uterine size in 40 females with TS (152). It was reported that uterine size was significantly affected by height and weight. However, Paterson, Snajderova and Bakalov found no correlation with height and uterine length in their studies (58, 143, 150). In my study height had no effect on final uterine size and, in those with TS, and the use of GH had no effect on uterine parameters.

In a subgroup, serum oestradiol levels were shown to be positively correlated with uterine size suggesting that continued adequate oestrogen dosing is

necessary and that this dose response curve for oestradiol could be exploited to increase the size of a small uterus prior to fertility treatment.

The strengths of my study are good participant numbers from different diagnostic cohorts and minimal bias in ultrasound findings as ultrasound was performed by a single operator using a standardised protocol and technique. I am aware of several limitations of this study. Clinical data was collected retrospectively and primarily by patient recall and therefore it was not possible to ascertain uterine size data prior to the initiation of therapy. I appreciate that those in the reference group were older than those in the diagnostic groups, and although age was not a predictor of uterine size, I cannot rule out the possibility that some of the differences are age related. In addition, there was no standardised method of induction of puberty to allow comparison between treatment groups and also the current maintenance HRT varies in type and dose which may mask the effect of the pubertal induction oestrogen. Furthermore, serum oestradiol was only measured in a subgroup of those taking HRT containing 17β oestradiol.

In summary, I have demonstrated that early onset oestrogen deficiency is associated with compromised uterine growth regardless of diagnosis. It may be that this observation is accounted for by inadequate oestrogen replacement doses as evidenced by an association between serum oestradiol and uterine dimensions. With higher oestrogen dosing in adults, it may be possible to normalise uterine volume in the majority and further studies are required to confirm this suspicion and the impact of uterine size on its function in pregnancy.

STUDY 4 DISCUSSION - VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM.

In this prospective cohort study, I set out to explore the variability in response to exogenous oestrogen in those undergoing puberty induction treatment. Despite a standardised treatment protocol there was marked variation in the increase in E2 concentrations and change in oestrogen target tissue responses over the 8-month treatment period. In the absence of a routinely available sensitive oestradiol assay, transverse uterine measurement was most closely correlated with oestrogen concentrations and could be used clinically as a surrogate marker to measure dose response. Whilst E2 levels determined uterine volume, it did not predict breast volume change, which was affected by age of treatment initiation. 3D breast volume assessment was correlated with Tanner staging and may provide a clinically useful, objective measure for breast growth.

Variation in serum E2 concentration, despite the same dose, was also seen by Ankarberg-Lindgren et al. (134) and can be accounted for by several factors. Adiposity was not correlated to E2 concentration in my study group suggesting that this variable does not affect drug absorption and delivery. Furthermore, oestradiol absorption is not thought to be affected by the cutting of the patch to manipulate the dose or storage temperature (135). As an adhesive matrix patch, it is designed to release a steady daily amount of 17β-oestradiol but the reliability of this may vary. Whilst some have demonstrated stable serum E2 concentrations throughout use (137, 138) others have suggested that there is fluctuation with peak and trough levels between dosing intervals (136).

The prepubertal uterus is tubular and grows not only in length but also transverse and anterior posterior diameter to obtain a mature configuration (18). In spontaneous puberty, uterine volume has been shown to correlate with E2 concentrations and breast Tanner staging and its growth continues beyond menarche and full breast development (18, 20, 46). Whilst I did not evaluate final uterine maturity, reassuringly during the initial months of pubertal induction treatment in my study, the transverse diameter grew in a similar fashion to those undergoing spontaneous puberty. In comparison with reference data from Hagen et al. (46), the transverse uterine diameter at each Tanner stage fitted within the normative range suggesting that later onset pubertal induction does not compromise early uterine growth, which is consistent with other reports and seen in study 3 (144). Interestingly, Tanner stage 1 measurements were comparable, suggesting that early prepubertal uterine growth occurs in the absence of high circulating oestradiol levels and maybe related to IGF-1, GH or adrenal androgens (46). Thereafter there was a drop off of some measurements at Tanner stage 2, reflecting those who were oestrogen naïve at treatment initiation and the deviation from normal physiology.

Historically, uterine dimensions have been used less often for assessment of puberty compared to breast staging and bone changes. Some studies have suggested that adult uterine shape can be attained with exogenous oestrogen treatment (124, 144, 148) whilst others have reported poor uterine development despite replacement (50, 58, 116, 143, 150). Whilst my study did not explore the variation in response based on aetiology, it is commonly reported that those with TS may have poorer uterine response. This may be secondary to inherent problems with uterine function including collagen deficiency or lack of X-linked genes regulating endometrial receptivity and responsiveness (165, 166, 339). In

this study, however, the two 2 females with TS showed a similar response in both uterine and breast growth to the other subjects (data not shown). Variability in uterine and breast response with fixed TD oestrogen, even in those with the same diagnosis, was also been reported by Nabhan et al. (124) suggesting other factors are also implicated.

During the initial 8 months of exogenous oestrogen treatment, the uterus appeared to be a very responsive end organ to oestrogen treatment with uterine dimensions demonstrating a significant rate of change correlating to the increasing E2 levels. My study demonstrated that uterine length was not a useful marker and did not correlate to rising E2 concentrations compared to transverse dimension and uterine volume. Whilst a uterine length of 65mm has been cited in many papers to represent uterine maturity (124, 143, 147), based on the normative data by Griffin et al. (71), this measurement has many limitations in practice due to the soft anatomical landmarking of the uterine body and cervix. I propose that the transverse uterine measurement should be used to monitor oestrogen response during treatment because it was most closely associated with E2 concentrations, develops in line with known reference data and also, as it is relatively simple to measure, it allows the ultrasound method to be focused. Transverse uterine diameter is also closely associated with uterine volume which might be considered to be the most important clinical parameter to indicate optimal development for future fertility.

In 8 months of treatment the majority of subjects displayed modest change in differential breast volume, which is consistent with spontaneous puberty. Breast budding is usually the first external sign of puberty, but full breast development takes several years. In the context of pubertal induction, studies have demonstrated development to Tanner stage 4 after an average of 2 years of

treatment (82, 115, 118). An appreciation that the breast tissue may progress more steadily than the uterus, at a variable pace, and likely later in the timeline of pubertal induction treatment is vital. In my study it is apparent that the breast develops at a slower pace than the uterus during the initial phase of exogenous oestrogen treatment and change in breast volume did not correlate with increasing serum E2. Therefore, attention should be paid to the uterus, rather than the breast development, in the early months of treatment to identify slow puberty progression. Whilst Tanner staging remains a clinic standard, 3D breast imaging providing a continuous measure may have its merits. With correlation between Tanner staging and 3D breast volume seen, this is a novel and promising experimental tool, and may have wider applications including shape analysis.

In my study, age of treatment initiation affected the breast development but not uterine size at 8 months. This may support the concept of a 'window' for optimal breast development and sensitivity of breast tissue to oestrogen exposure being greater at an earlier age. Late onset of puberty induction (over the age of 14) has been shown to compromise final breast development which was not related to dose of oestrogen (115, 116). In my cohort, during 8 months of uniform treatment, the volume change for the majority was small. Out of 13 females who underwent 3D breast image acquisition 10/13 76.9% were over the age of 14. The female who achieved the greatest uterine length during the study period, also reached Tanner breast stage 4 with the largest change in breast volume during the study duration, was also the youngest participant, suggesting age of treatment initiation is likely to be key for development potential.

Most studies examining puberty induction are with females with TS. In contrast my study is heterogenous with varying causes of hypogonadism. The underlying

diagnosis may affect response. At presentation some of the participants had detectable oestrogen levels which was not attributed to adipose production of oestrogen. This was seen in those with HH and 17 beta hydroxylase deficiency suggesting that perhaps the oestrogen production of the ovary is not zero in these diagnoses. This is a subtlety revealed by the use of the sensitive oestradiol assay in this study.

Suppression of gonadotrophins may be an indirect marker of oestrogen effect (127). FSH appeared to be more sensitive in this regard compared to LH. Whilst gonadotrophins may act as a useful marker in treatment monitoring, their clinical use is limited as they are only applicable to those with hypergonadotrophic hypogonadism and not to those with gonadotrophin deficiency.

Current practice often relies heavily on anthropometric measurements, but anthropometric measurements did not correlate with oestradiol concentration in my study. I found no difference in body composition measures, body fat or BMI, after 8 months of treatment, which is in contrast to other papers suggesting an increase in body fat over the pubertal years (41-43). These papers have examined this over several years and an obvious reason why my results may not follow the same pattern is the short duration of follow up. Interestingly, Vink et al., examined fat mass in girls between Tanner breast stages 1-5 and although they found that fat mass increased in all Tanner stages, between stages 2-3 the accumulation in fat mass was not significant. The authors hypothesised that between Tanner stages 2-3 there is also linear growth leading to a high metabolic state (43). During my study period 37% progressed from Tanner stage 2-3 which may also contribute and there was an increase in height in 8 months of treatment. Whilst it most likely related to the short follow up duration, other papers have also not identified a change in body fat during puberty (340) .

A further consideration is that exogenous oestradiol treatment may herald different metabolic effects that endogenous hormonal production. Reinehr examined a cohort of females with TS and found that compared to those who went through spontaneous puberty there was no difference in BMI (44). My study used a transdermal oestrogen preparation, but other studies have not identified a difference in body composition outcomes between different oestrogen preparations (44, 124, 129, 131).

One of the goals of pubertal induction treatment is attainment of adult height in line with genetic potential. I found that during the study period there was a significant change in height, but there was variation in the response between individuals. The growth potential may be related to the varying underlying pathology, the use of growth hormone or the age at presentation.

During the study period there was a significant increase in lumbar spine bone density indices, whereas those of the hip did not follow the same trend suggesting that spine (trabecular bone) is more oestrogen sensitive compared to hip (cortical bone). In studies that have examined the effect of oestrogen deficiency in postmenopausal women, trabecular bone appears more sensitive to decreasing oestrogen levels, which may be secondary to the fact that trabecular bone contains both ER subtypes and also has a larger surface area compared to cortical bones. Furthermore, during puberty, due to oestrogen, excess calcium is stored in increased amounts in the trabecular bones (341).

In the study design I considered it ethically appropriate to hold a standardised dosing for 8 months so as not to impair progress for subjects who may require a higher dose schedule. Thereafter there was a clinical need to have divergence in dose, with some participants continuing on the same dose and others having an

increase in the dose, based on age and pubertal stage. Therefore, ongoing comparisons of variability in response would not have been possible. There is not a unified approach to pubertal induction treatment and there was no clear protocol for decision making regarding the titration of dose after 8 months of treatment. I found that age appeared to have the most influence of decision making, although this was not statistically significant. In my study cohort however 87.5% of participants were 14 years or older with treatment initiation. There is little data regarding the dosing practice in those that present later which is perhaps why decision making was based on clinician experience and clinical acumen. I chose to start on 12.5mcg, which is in line with the paper by Lee et al. (122) and Gawlik et al. (115) but more conservative than the recent review by Howard et al. who recommend a start dose of 25mcg transdermal patch in those who start treatment when they are 15 years or older (5). In neither of these papers have the authors recommended the interim dose of 37.5mcg and recommend changing directly to 50mcg from 25mcg. What is interesting, and reassuring is that the dosing choice did not affect the trajectory of puberty development between 8 and 12 months. The higher dose however may precipitate earlier break through bleeding, and in my cohort only 15.3% achieved a uterine volume above the 5th centile of the normal range and only 26.7% reached Tanner breast stage 4 respectively by 12 months and if progesterone is added too early this this may ultimately compromise development. This is something to explore further in terms of incremental change in those who present late and the long-term outcome.

This study, in addition to the results in study 3, highlight the key message that uterine development should consist of ultrasound assessment using all uterine size parameters rather than only using uterine length to define full uterine

maturity. Whilst 46.1% achieved a uterine length above the 5th centile of the normal range only 15.3% achieved a uterine volume above the 5th centile of the normal range after 12 months of treatment. Cessation of unopposed oestrogen too prematurely may ultimately affect full uterine development.

I recognise and that clinic visits were not synchronised with the timing of patch change, which may have affected the E2 concentration. Treatment compliance may account for some of the variability in E2 concentrations and response, and whilst I endeavoured to limit the impact of this by single observer verbal discussion at each visit, it is accepted that the decision to adhere to treatment is multifactorial and accurate rigorous assessment is a challenge with no gold standard (342). I appreciate that a longer follow-up period would provide more clarity about final outcome, but the aim of the study was to examine variability in response to exogenous oestrogen rather than to establish the plateau of treatment. Exploring the impact of aetiology on treatment response would be very beneficial for clinical decision making, and may contribute to the variability of outcomes, however the small sample size in each subgroup would make analysis limited and therefore not meaningful. I appreciate that 3D breast imaging of a larger number of participants and comparison to a control group of those with spontaneous puberty would be required to further evaluate its clinical use. This and its use in analysing breast shape are the scope for future research.

In conclusion, I have demonstrated a large variation in response to exogenous oestrogen in those undergoing puberty induction treatment. While completion of pubertal induction usually takes approximately 2- 3 years, the duration and pace may vary depending on several factors. In the routine clinic setting the dose of oestrogen may be chosen based on body weight, age or growth pattern (82, 120). Response to treatment should be monitored at regular intervals with assessment

of many endpoints, including breast and uterine growth and oestradiol concentrations, every 3-6 months. In the early stages of treatment routine oestradiol assays are not suitable to monitor response due to oestradiol levels falling below the limit of detection and therefore transverse uterine measurements may be used to monitor tempo and guide individualised dose adjustments.

STUDY 5 DISCUSSION - QUANTITATIVE ASSESSMENT OF BREAST VOLUME USING 3D IMAGING IN TRANS FEMALES: A PILOT STUDY

In this pilot study, I set out to explore the application of 3D breast imaging in trans females undergoing gender-affirming hormone treatment. Changes in breast volume were seen after 6 months of treatment and calculation of breast volume was possible with 3D breast imaging. Variability in oestradiol concentration was seen after 6 months of treatment implying that imaging may be more informative than blood tests especially in the absence of sensitive oestradiol assays. 3D breast imaging may be a useful adjuvant to monitoring in this cohort and a tool for future research to help ascertain factors associated with optimal development.

This represents a pilot study to assess the 3D breast volume technique and the feasibility in the adolescent cohort and I now hope to widen the research to include more participants. Longitudinal volume assessment would provide more guidance on treatment and understand the natural history of breast development (216). Many individuals in this cohort are unhappy with their breast development and therefore turn to surgical augmentation (220, 223). Understanding factors associated with breast development may prevent dissatisfaction and optimise treatment with medical intervention alone.

STUDY 6 DISCUSSION - FERTILITY AND PREGNANCY OUTCOMES IN WOMEN WITH TURNER SYNDROME AND PREMATURE OVARIAN INSUFFICIENCY

In this study I set out to examine fertility and pregnancy outcomes in women with TS and POI from a large single centre cohort. I show that overall pregnancy in women with TS whether by OD or spontaneously conceived, carries obstetric risks above general population. Reassuringly there were no recorded cardiac events in this cohort. In spontaneous pregnancies 5 cases of vertical transmissions were identified. Women with TS who had OD had a higher rate of miscarriage compared to women with POI using OD.

Whilst TS OD pregnancy is associated with increased rates of LSCS delivery and SGA compared to TS spontaneous pregnancy, risk for other outcomes with OD were not different from those in spontaneous pregnancies suggesting that there is not an excessive summative risk of TS with OD.

The extent of the X chromosome loss usually dictates the severity of the TS phenotype and those requiring OD usually have more severe phenotypic parameters associated with TS. I found that women with TS undergoing OD pregnancy had increased prevalence of monosomy, primary amenorrhoea and thyroid pathology compared to those with spontaneous conception. This may explain the additive effect of TS and OD and the excess risk above spontaneous pregnancy for some outcomes.

Whilst spontaneous puberty occurs most commonly in those with mosaicism, spontaneous conception occurred in one woman in this cohort with 45, X, which has also been reported in other papers (93, 94). This suggests that the cell line may be discrepant in the peripheral blood to that of the ovary (94). With the

possibility of spontaneous pregnancy, clinical consultation should include contraceptive advice to prevent unplanned pregnancy.

In women with TS undergoing OD, I found a pregnancy rate per cycle of 53.6% which is comparable to other papers of women with TS where the rates vary between 33 - 57% (247, 248). Encouragingly, the live birth per cycle started in this cohort of 31.1% in those with TS is comparable or higher than other reports of 3.2%, 17.9% and 27% and 33% (93, 240, 247, 258). I found that the pregnancy rate per cycle was similar between TS and POI, as has been reported by other groups comparing pregnancy rates to oocytes recipients for other indications (166, 258, 339). I have demonstrated a lower live birth rate per cycle started in women with TS undergoing OD compared to POI. This was not accounted for by abandoned cycles. One explanation may be deficient uterine and endometrial integrity in those with TS affecting optimal embryo implantation (256).

Several papers have identified an increased risk of miscarriage in women with TS regardless of mode of conception (90, 164, 243, 244, 247, 251, 256, 258). I identified a higher miscarriage rate in women with TS compared to both POI and general population data. In women with TS and OD pregnancies the rates of miscarriage in the literature vary from 25% to 44% compared to the rate in this study of 40% (93, 164, 244, 247, 248). The higher miscarriage rate witnessed for both TS and POI with OD, may be coupled to the higher prevalence of thyroid pathology and autoimmunity associated with these diagnoses (82, 103, 120). Despite the occurrence of hypothyroidism being similar in those with TS and POI, there was still an elevated miscarriage rate and lower live birth rate in those with TS OD, suggesting that mechanisms other than thyroid dysfunction may be implicated, perhaps uterine factors. Earlier loss of ovarian activity in those with

TS compared to POI, leads to a higher proportion with primary amenorrhoea necessitating pubertal induction treatment. Despite seemingly sufficient exogenous oestrogen therapy, uterine size is often reduced compared to women with normal puberty as seen in Study 3. However, the increased risk was also seen in those with secondary amenorrhoea and those with spontaneous conception, suggesting other factors affecting uterine function such as poor endometrial development, deficient uterine vascularity, collagen deficiency, lack of X-linked genes regulating endometrial receptivity or poor epithelial integrity with lack of tight junctions (165-167, 169, 339).

The miscarriage rate in women with TS experiencing spontaneous pregnancy in this study was 32.4%, which falls in the ranges previously reported between 22.8 and 67.3% (90, 93, 164, 244, 256). In the study I found a comparable rate of miscarriage in those with TS undergoing OD or spontaneous pregnancies which challenges the hypothesis previously suggested that oocytes from women the TS carry an increased risk of aneuploidy (90, 164).

Cardiac health is a particular focus in women with TS considering a pregnancy. Interestingly, no cardiac event including aortic dissection occurred during the antenatal or post-partum period, which are events reported in the literature (100, 164, 240, 241). The 2024 published TS clinical care guidelines outline that an echocardiogram should be completed within 2 years before conception with calculation of the ascending ASI (120). If the ASI >2.5cm/m^2 or between 2.0-2.5cm/m^2 with compounding cardiac pathology such as bicuspid aortic valve, coarctation of the aorta, HTN, elongation of the transverse aorta or history of aortic dissection then pregnancy should be avoided. During pregnancy, as aortic dissection has occurred in women with normal pre-pregnancy cardiac status (240), an echocardiogram should occur at least once at approximately 20 weeks

of gestation in the absence of any identified risk factors or every 4-8 weeks during pregnancy and up to 6 months postpartum if other risks present (82, 120).

Hypertension during pregnancy is more prevalent in this TS cohort (both OD and spontaneous pregnancy) compared to spontaneous conception population data, however my study did not find a difference in HTN between the modes of conception in TS, which is in contrast to previous papers suggesting a summative risk of OD and TS (100, 240, 246, 247). Other more recent papers have found comparable levels between the conception groups (93, 241). Reassuringly, whilst women with TS undergoing pregnancy still pose a higher cardiovascular risk, the rate of HTN pathology in those with TS and spontaneous pregnancy (12.5%) and those undergoing OD (16.7%) in this study was on the lower side of the other quoted rates in the literature 11 - 20% in those with spontaneous conception (93, 243) and 15 - 62.5% for OD (94, 100, 240, 244, 247, 248). Furthermore, there was not a significant difference in the rate of HTN pathology between TS and POI OD pregnancies, which is in contrast to other papers (246, 247). There may be many variables affecting the prevalence of HTN including local treatment thresholds and decision making by the local team.

Importantly in this large cohort of TS pregnancies, I did not demonstrate any significant cardiac events including aortic dissection either during the antenatal or post-partum period, which is a sequelae reported in the literature (100, 164, 240, 241, 246).

The more favourable cardiovascular outcomes detailed in my study may reflect the early adoption of pre-pregnancy cardiovascular surveillance guidelines at UCLH. Cardiology assessment dated back to pregnancies in 2001 and echocardiogram in 1999. In this TS cohort of women seeking fertility the

prevalence of cardiac pathology was lower than the cited rate in the overall TS population of 25-50% (93, 99) suggesting that a degree of case selection had taken place.

The rate of LSCS was higher in women with TS irrespective of mode of conception and when compared with either population reference data or the POI cohort, consistent with previous reports (6, 11, 12, 13, 34, 39). The increased rate of LSCS in women has been linked to multiple factors including short stature and feto-pelvic disproportion (243, 249), avoidance of exacerbation of underlying cardiovascular disease, expedited delivery due to maternal/fetal indications or patient preference. Whilst many women with TS will have a LSCS and, LSCS may be the preferred mode of delivery for some patients and obstetricians, it should not be overlooked that LSCS also poses risks and is itself associated with haemodynamic changes (82). Therefore, LSCS should not be the default and vaginal delivery should not be excluded. Notably, 29% of women with TS achieved a vaginal delivery so a trial of labour may be possible depending on the multidisciplinary team review.

Pregnancies in women with TS have been shown to be associated with increased rates of prematurity and low birth weight (94, 100, 230, 240, 243, 244, 247, 248). This may be placentally driven (230) or alternatively, the HTN disorders may cause intrauterine growth restriction and in turn precipitate premature delivery. Concurring with this I demonstrated a higher rate of SGA in TS OD pregnancies compared to TS spontaneous conceptions and general population reference data. Premature delivery, however, was not found to be different between TS spontaneous and TS OD pregnancies which is in contrast with some earlier studies (100, 247), but not others (240, 241), possibly reflecting differences in

active management of complications minimising the need for expedited delivery and iatrogenic prematurity.

I demonstrated a predominance of female offspring following spontaneous conception in women with TS. This is to be expected as a 45,Y0 karyotype would not result in a viable pregnancy. Other authors have also commented on this finding with calculated M:F ratios between 0.37 and 0.84 (93, 243, 249).

Vertical transmission of TS is a known risk in women with spontaneous conception. This has been poorly documented in past literature and as systematic screening has not been undertaken the real incidence remains unknown (90, 92, 93, 243, 253). In the study by Birkebaek et al., chromosomal analysis was completed for 25 of the 64 (38.4%) children born following spontaneous conception in those with TS. Five girls (5/32 15.6%) were found to have chromosomal aberrations consistent with TS (33). Tarani et al. reported 13 spontaneous pregnancies in 6 women with TS and out of 8 live births, 2 daughters had TS (90). Bernard et al. reported the outcomes from 56 spontaneous pregnancies in women with TS and in those 30 children born, TS was detected in 2 daughters (6.6%) and trisomy 21 and 13 in others (243). In the series by Calanchini TS was diagnosed in one female in adulthood as was found to have the same karyotype as her mother (93).

I demonstrated a higher rate of vertical transmission of X chromosome anomalies than that previously highlighted in the literature partly because of one family passing on mosaic an X;Y translocation over three generations. This karyotype has been shown to be prone to transmission previously (254). As consequence of my findings I recommend that preconception consultation should include the discussion regarding the option of prenatal or antenatal genetic counselling.

Elective single embryo transfer (eSET) should be standard best fertility practice and, in those with TS planning OD treatment, eSET should be particularly advocated to minimise the additional maternal and perinatal morbidity and mortality associated with multiple pregnancy (319, 320). The better maternofetal outcomes in this study maybe because analysis was limited to singleton pregnancies, however the multiple pregnancy rate in this TS OD cohort is low at 13.1%. Given my data covers several years predating the Human Fertilisation Embryo Authority (HFEA) 'One at a Time' campaign in 2007, the rate of multiple pregnancy is encouraging, suggesting that patient education and adherence to policy is being met. Historically eSET was less common, the paper by Chevalier et al. reported a double and triple embryo transfer of 40 % and 18 % respectively (100) and multiple pregnancy may have contributed to increased adverse outcomes in earlier papers. Hagman et al. found that in women with TS and OD pregnancies the risk of preterm birth and SGA was significantly higher in twin pregnancy vs singleton (preterm birth 66.7% vs 8%, SGA 72.2% vs 8.8%) (240).

In the general population, a common indication for OD is to circumvent subfertility associated with advanced maternal age. Maternal age especially over the age of 35, is an independent predictor for many obstetric outcomes (343), and therefore may be a contributing factor to the adverse obstetric outcomes associated with oocyte donation (247). In my study age was not a contributing factor to adverse outcome between the OD and spontaneous cohorts in either diagnostic group. Even though those accessing OD (both TS and POI) were older than those with spontaneous pregnancy, their median age at pregnancy was less than 35 years of age.

Understanding the factors predictive of those with idiopathic POI who may have resumption of ovarian activity would be beneficial clinically for patient counselling

and there have been several papers examining this (304, 305, 307, 308, 313). In the literature those with primary amenorrhoea are less likely to demonstrate ovarian activity and this was consistent with my data with only 1/15 (6.7%) having a spontaneous pregnancy. In this POI cohort I concentrated on pregnancy rather than other markers of ovarian activity. However, I appreciate that exploring the clinical features in the much wider cohort of women with POI, examining prognostic factors for ovarian activity, would be very valuable. The small cohort for this study did not allow me to produce a predictive model for spontaneous pregnancy.

There was no difference in miscarriage rate between OD and spontaneous pregnancy or compared to general population data, which is reassuring in term of oocyte quality of those with natural resumption of ovarian function. Bidet et al. reported a miscarriage rate of 19% compared to the miscarriage rate of 25% in this study (304). There is little data examining maternofoetal outcomes in patient with POI who have spontaneous pregnancy, and most is based on single case reports (316, 317). Whilst these case reports have detailed uncomplicated pregnancies it is not possible to conclude risk assessment confidently given the small numbers. To the contrary, I found increased obstetric risks for some outcomes in those with POI and spontaneous pregnancy, namely HTN, GDM and SGA. One larger series examining the outcomes of 21 spontaneous pregnancies in 15 women with idiopathic POI found an increased risk of prematurity compared to general population data (304). Whilst this study prevalence of preterm birth of 14.3% was not significantly different from the prevalence in the general population data, it does appear higher than 4.7%. From the small data set this this information should be used with caution as to the true additional risk in this cohort.

Outcome data for POI and OD outcomes is usually extrapolated from the general OD data. Compared to the meta-analysis data by Storgaard et al. examining singleton OD pregnancies, in this POI OD cohort the prevalence of HTN was 20.8% and LSCS was 57.1%, which both fell within the ranges reported (HTN 13 - 39.3%; LSCS range 31.4 - 85%). The prevalence of GDM was 16.7% which was higher that the reported range of 0-13%. The SGA rate in this study was 27.7% and higher than the reported prevalence of 0-9.3%. The preterm birth rate was 8.5% compared to the range of 10 – 24.3% (230).

One hypothesis for additional risk in those with POI may be linked to autoimmune pathology in this cohort and perhaps uterine factors affected by HRT. At present, especially in the spontaneous pregnancy cohort, the outcomes are not conclusive and is certainly the scope for future research.

In those with TS, the uterine size parameters were significantly smaller in those undergoing OD compared to those with spontaneous conception. This would be expected as the majority with OD pregnancy had primary amenorrhoea with pubertal induction treatment, which may have impact final uterine size and maturity as reported in study 3. There was no significant difference in uterine parameters between the TS and POI. I speculate that the result may not be accurate, as in most cases, the pelvic US were completed for those with POI at the time of diagnosis. Therefore, there would be state of oestrogen deficiency before being established on HRT. It could be hypothesised that the uterine size would be smaller as extrapolated by the reduction in uterine size in post-menopausal women (55, 65), and therefore, comparison between the diagnostic groups and analysis of the impact of uterine size in those with POI may not be reliable.

I recognise that Information was gathered mainly by patient recall which may have led to some inaccuracies, however the reliability of self-reported pregnancy outcomes has been investigated by other groups, with reassuring results (344). All karyotypes were not available as the paediatric data was not accessible in some cases and I was not able to obtain the karyotype of two of the daughters born with TS as the mothers were lost to follow up. Furthermore, locating all the antenatal cardiac data is challenging as antenatal care took place in other centers, local to the patient's home. Not all uterine data information was complete, and the results may have been affected as the ultrasound was not completed by a single observer and often measurements were placed on stored images.

In conclusion, women with TS considering pregnancy should receive comprehensive education regarding pregnancy and understand that overall pregnancy may pose a higher risk than those without TS. Pre pregnancy counselling for those with TS who have the possibility of spontaneous pregnancy needs to address the possibility of vertical transmission. Overall, my results of the risk of pregnancy are reassuring and it is possible that improved cardiovascular screening and obstetric management are already showing the benefits of guidelines for adult care of women with TS (82, 120).

DISCUSSION OVERVIEW

Spontaneous female puberty is a highly variable process and therefore pubertal induction should be no different. There is a complex interplay of several factors that affect the response to oestrogen and its culmination in the maturity of secondary sexual characteristics. While guidelines recommend that treatment should be individualised to ensure optimal clinical care there is little evidence from research to inform on this process. My studies have provided greater insight into puberty transition with exogenous oestrogen and added to this clinical field.

I have demonstrated that we should move away from categorical developmental markers and towards continuous parameters for breast assessment and uterine growth, with the latter to be considered the gold standard early predictor of oestrogen response.

Uterine development is a fundamental part of pubertal development and can be used as an excellent marker of oestrogenisation and efficacy of treatment. Many previous papers have paid attention to uterine length and FCR (17, 60) but I demonstrate that ultrasound assessment of the uterus should not only encompass a single measurement of uterine length but report all the uterine dimensions. My work suggests that the uterine transverse measurements may be the most effective parameter as a marker of oestrogen sensitivity. Therefore, an appreciation of the uterine morphology, as a whole, is vital to monitor of treatment. Through examining the different uterine parameters and their individual inter and intra observer variability I was able to produce an optimal protocol for transabdominal ultrasound. The standardised protocol generated excellent reproducibility for TAUS, when completed by experienced practitioners providing reassurance for its first line use in serial assessment of uterine size.

The results suggest that interchanging between imaging modalities should not be advocated.

I developed a novel technique for quantitative 3D breast measurement in those undergoing exogenous oestrogen treatment. Although the method is still in its infancy and not yet rolled out into routine clinical practice, it may serve as a promising technique and prove a worthwhile adjuvant clinical tool complementing the established but subjective Tanner staging. 3D breast assessment may also provide valuable insight into the limited knowledge base regarding the natural history of breast development in trans females.

My work demonstrates that, with exogenous oestradiol treatment, there is great variation in the increase in E2 concentrations, uterine dimensions and change in breast volume between individuals. My data suggests differences in oestrogen sensitivity may be key and expands the knowledge of the consequences of this variation with implications for treatment dosing. Given this variation, rate of change can be assessed more easily with a continuous variable compared to the interval variable of Tanner breast staging. Early dosing decisions will inevitably be delayed due to the lag time in changes in Tanner breast stage. Utilising the more subtle fluctuations based on serial uterine parameters and 3D breast volumes change allows more proactive dose titrations centred on the individual response. This highlights the need for significant shift in practice, moving away from standardised protocols for all with oestradiol dosing based on single anthropometric parameters or a fixed regime. I also highlight the need for a more tailored approach for the older adolescent requiring pubertal induction who often have different clinical priorities than girls who require treatment earlier. I suggest that the application of the current early pubertal induction protocols cannot be applied as default in the older adolescent cohort.

Years after completing puberty, many women will consider assisted reproduction to help achieve a pregnancy and adequate uterine development is essential for implantation and reproductive health. I have demonstrated that many women do not fulfill their potential uterine growth with oestrogen therapy and therefore meticulous optimisation of uterine development is essential.

I provide outcome data on fertility and pregnancy in women with TS with both OD and spontaneous conceptions from a large single centre cohort. Uniquely I compared data with women with POI undergoing pregnancy which has not been seen in the literature before. I show that TS pregnancy carries obstetric risks and therefore women with TS, considering pregnancy, should receive comprehensive pre pregnancy counselling and optimal obstetric care, which will shape clinical practice. Little has been published on the obstetric outcomes in women with POI and spontaneous conception and I propose that spontaneous pregnancy in women with POI should not assumed to be low risk until further work is completed.

I am aware that a limitation of my work is the smaller sample number which in some cohorts did not allow for extensive statistical analysis. Nevertheless, given that these are uncommon diagnoses with recruitment also pressurised by the timescale of the research programme and the necessary attendance at both Paediatric and Reproductive Endocrinology departments, this body of work represents a significant advancement in the clinical knowledge base with sizable numbers, often surpassing previous cohorts. I recognise, that at UCLH, I was in a unique position to recruit to this type of study and the difficulty of recruitment is one reason why there is so little research on the topic. Furthermore, as UCLH is a tertiary centre with dedicated clinics we have clinicians with expertise in ultrasound scanning, MRI and medical physics permitting this research.

In conclusion, the heterogeneity and duration of puberty changes make the study of outcomes a challenging but nonetheless rewarding pursuit. As randomised controlled trials are difficult to perform in this area, most information leading to refinement of treatment protocols will come from close observation and organised data collection from real world experience. Taking all of the information from the studies included in this thesis, one is led to the conclusion that induction of puberty is a specialist topic that spans both paediatric and adult medicine necessitating a dedicated service of uninterrupted care through to fertility.

CHAPTER 10

FUTURE WORK

Whilst my work has provided much insight into the missing knowledge gaps within the field, this has only brought about new questions that will be the motivation and scope of future work.

The use of ultrasound is commonplace and hopefully will become a routine clinical tool to monitor oestrogenisation and guide treatment. Ultrasonography is still limited, somewhat by operator factors and placement of anatomical landmarks, especially if a standardised protocol for measurements is not used. Newer modalities are being developed to measure volume using ultrasound, which may provide more accurate and reproducible results such as VOCAL™ (Virtual Organ Computer-aided AnaLysis). This has already been used to measure uterine volume using the transvaginal approach and in the future I would wish to explore using VOCAL™ (46, 345) as an alternative method for obtaining uterine volume in this cohort via the transabdominal route. Furthermore, there has been the application of other markers to assess puberty progression including the assessment of Doppler assessment of uterine artery pulsatility index. It has been demonstrated in the context of spontaneous puberty that there is a significant reduction in pulsatility index during puberty (55, 56) and it would be interesting to examine this parameter in the context of puberty induction.

Ovarian ultrasound parameters including size, volume and antral follicle number may be useful adjuvant markers in some females undergoing pubertal induction treatment and I will aim to incorporate these measurements when I expand the number of participants in the future.

Examining factors which influence final uterine size in the context of pubertal induction treatment in a retrospective fashion is hindered by several factors. It has been hypothesised that oestrogen treatment with transdermal patch would see a more favorable result in terms of uterine development and in the future a randomised controlled trial examining the outcomes in more detail would be beneficial.

As the 3D breast volume assessment is a new technique, I acquired the skills for image manipulation and volume calculation using personally developed methodology. As a result, interobserver variability could not be examined and therefore this will need to be included in further work. This will also be important to allow others to develop the skill set necessary so that, in the future, this maybe an assessment tool used routinely in clinical practice. Furthermore, I wish to document variability in breast development during spontaneous puberty to understand 3D breast volume change in more detail.

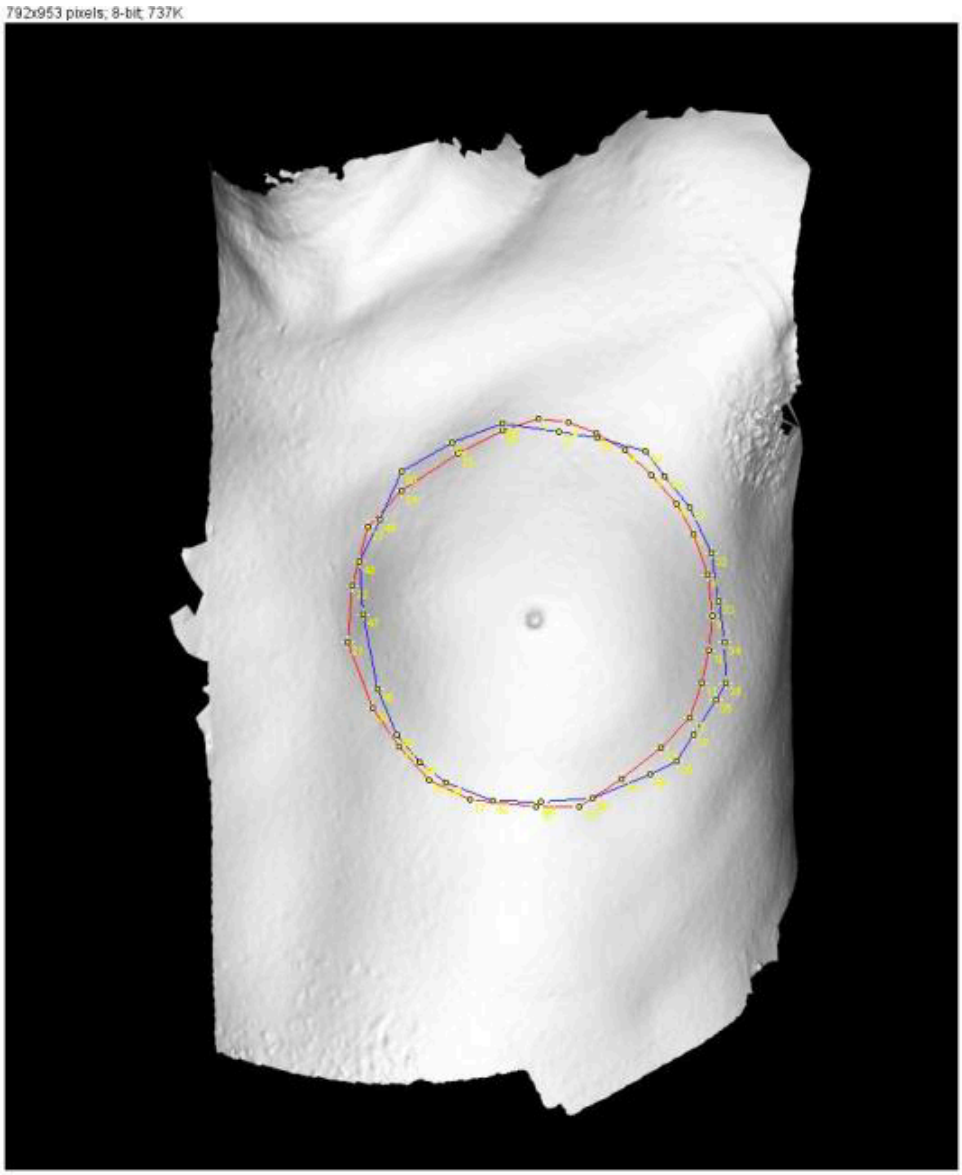
Breast development is a fundamental part of pubertal induction, and dissatisfaction regarding breast growth can stem from both breast size and shape. This may lead to significant psychological impact and be the motivation for many to turn to surgical intervention. Whilst it is dogma that abnormal breast shape can ensue from exogenous hormonal treatment there is no quantification tool to measure this and provide any scale regarding shape patterns and severity. To do this we need to move away from visual assessment with descriptive accounts based on clinician experience only and provide this ‘problem’ scientific underpinning. This will give patient and clinician greater understanding and confidence when discussing this with patients. My long-term aim is to compare breast shape analysis in women after puberty induction to those after spontaneous puberty

In order to complete shape modelling, I need to be able to parameterise the breast shape to identify common shape patterns. I initially completed manual selection of common landmarks on the breast, but this was hindered by reproducibility of point landmarks.

With the collaboration of the Principal Medical Physicist and the ongoing development of computer software we hope to be able to circumvent the problems with landmarking and have a new technique that we hope to trial in the future. This is the boundary model, whereby I am not delineating certain points but only the breast boundary where the breast starts to become evident on the chest wall. Multiple points can be placed to delineate the boundary as seen in Figure 10.1 and this will hopefully overcome the issue of needing to identify an exact point which may be affected by rotational image manipulation. The marks are placed on the boundary starting at an arbitrary point and the subsequent points are placed clockwise from this position until the boundary is closed. This requires much work to ensure adequate reproducibility and is far more complex that single point reproducibility.

Figure 10.1. Image demonstrating the 'Boundary Model'

Markers are placed on the boundary of the breast border.

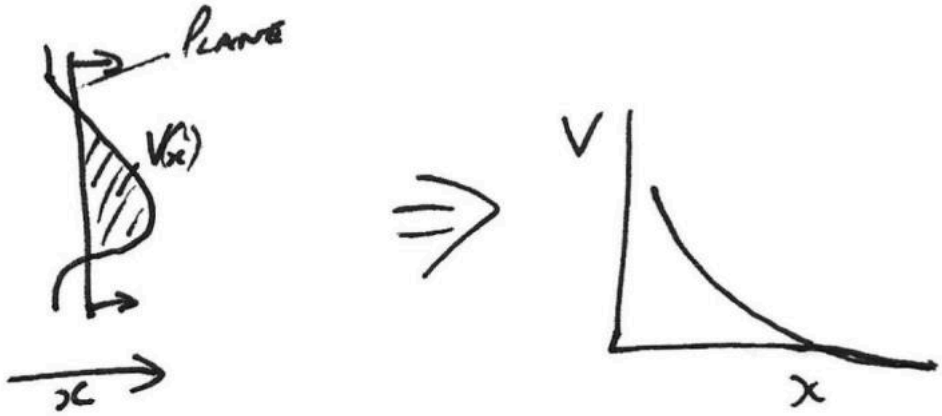


Once the boundary is in place and adequate reproducibility of the technique is demonstrated I will then go onto trial two different methods to try and produce a quantitative assessment of breast shape. The first is the 'Moving Plane' method whereby a 2D plane is placed on the breast boundary and the volume is calculated in front of this plane. The plane is then advanced forward at set amounts and a new volume generated. It is hoped that the shape of the resultant graph will provide information about breast shape as shown in Figure 10.2.

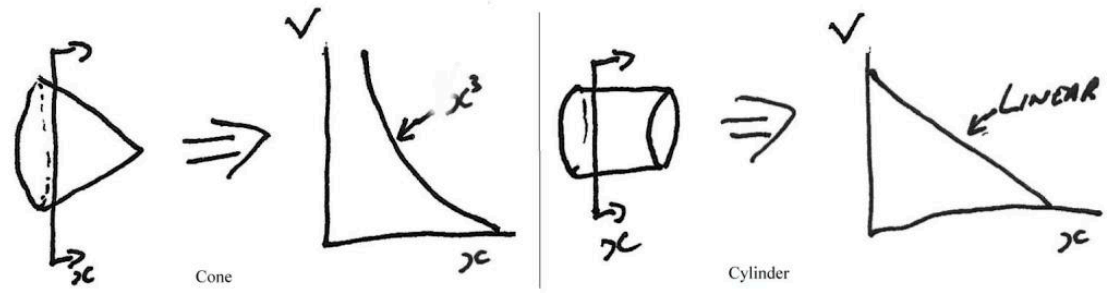
Figure 10.2. Figure demonstrating the graphs expected with the 'moving plane' method with the common breast shapes experienced

A. Round shaped breast and associated graph shape
B. Cone shaped breast and associated graph shape
C. Cylindrical shaped breast and associated graph shape

A



B **C**



This is a novel technique and has not appeared in the literature. Of course, these graphs at present are simplified but with experience, applying this technique to many breast images, there will be further understanding into the subtleties in the graphs exhibited and further analysis and quantification can begin. Furthermore, the technique and software can be validated by running the software of computer-generated cones and cylinders and also by warping the images to simulate participant movement and change in posture which may affect the breast boundary. Image warping may also be used to simulate the subtle changes in

shape we may expect to see clinically, to see how the graph shapes are affected and enhance our understanding of this.

The second technique that I will trial for shape modelling once the boundary technique is optimized is the Point Distribution Model. For this technique the breasts boundary and the point of the nipple are defined. Further points are then placed equidistant along the defined boundary. These points will be used as a basis of interpolation across the surface of the breast.

There is still much unknown about pubertal induction treatment, especially in those that present later and how to optimize treatment whilst maintaining psychological wellbeing is not known. My study only examined 12 months of treatment without evaluating the final outcome and certainly future work would explore treatment in its entirety. With faster tempos of dose change there is the increased risk of earlier breakthrough bleeding with incomplete uterine and breast development. The balanced between ongoing unopposed oestrogen treatment and ensuring endometrial health is a challenge and there is no unanimous agreement of how to navigate this clinical scenario. The paper by Howard et al. have suggested a reduction in oestrogen by 25% of the current dose (5), but in our practice we have also continued the same oestrogen dose with ultrasound endometrial assessment every 3-4 months or to give a single progesterone dose to induce withdrawal bleed prior to the continuation unopposed oestrogen. There is no published literature examining the different practices in more detail and would be the scope of my future research to understand this in more clarity.

My study has focused on pubertal induction in the context of those who present primary amenorrhoea and Tanner breast stage <2. Another cohort of females who require pubertal induction is those with secondary amenorrhoea and

incomplete breast development. These females are not oestrogen naïve and have already been exposed to endogenous progesterone. The variability in response to oestrogen and final outcome in this group would provide further understanding as to the impact of prior endogenous hormonal production. I did start examining this cohort in terms of breast and uterine development during my research time as part of routine clinical practice and assessment, however as this element did not feature in my initial research protocol there was no standardised treatment or inclusion criteria and assessment was based on their scheduled clinical appointment rather than fixed timings. Due to this heterogeneity I did not continue the recruitment process. This is certainly an aspect I would like to focus on again with a clear research protocol.

Breast development in the trans females is incredibly important for the majority, but little is known regarding optimal development. I have started the journey of further understanding with the application of 3D breast imaging during oestrogen treatment in this group, which may provide both qualitative and quantitative information. A future prospective study with the use of 3D breast imaging with larger numbers may allow expansion of knowledge in this field.

I propose that uterine factors both size and function may play a pivotal role underlying reproductive risk in TS, and the aim of future work will be to explore this further. Whilst I endeavoured to examine uterine size in those with TS and POI who had undergone fertility and pregnancy, there were several limiting factors associated with the retrospective data. My aim moving forward, to ensure this data is more robust, is to complete ultrasound assessment of the uterus using my standardised protocol myself. I will complete the ultrasound prior to fertility treatment and or the consideration of pregnancy. In addition, in those with POI, most scans were assumed to have been completed as part of the initial diagnostic

work up rather that prior to fertility treatment per se, when they were not established on HRT which may have also affected the results.

There is little published data regarding the obstetric outcome of spontaneous pregnancy in those with POI. I found some interesting results perhaps suggesting an increase in maternal and fetal risk. This data should be used clinically with caution at present as it is based on small number and therefore, I would wish to expand this data set to explore this in more detail as this may motivate a change in clinical practice and risk stratification for these patients.

In summary the scope for future work is both exciting and broad and my doctoral work only represents the tip of the iceberg in terms of the exciting new avenues my research interests may follow.

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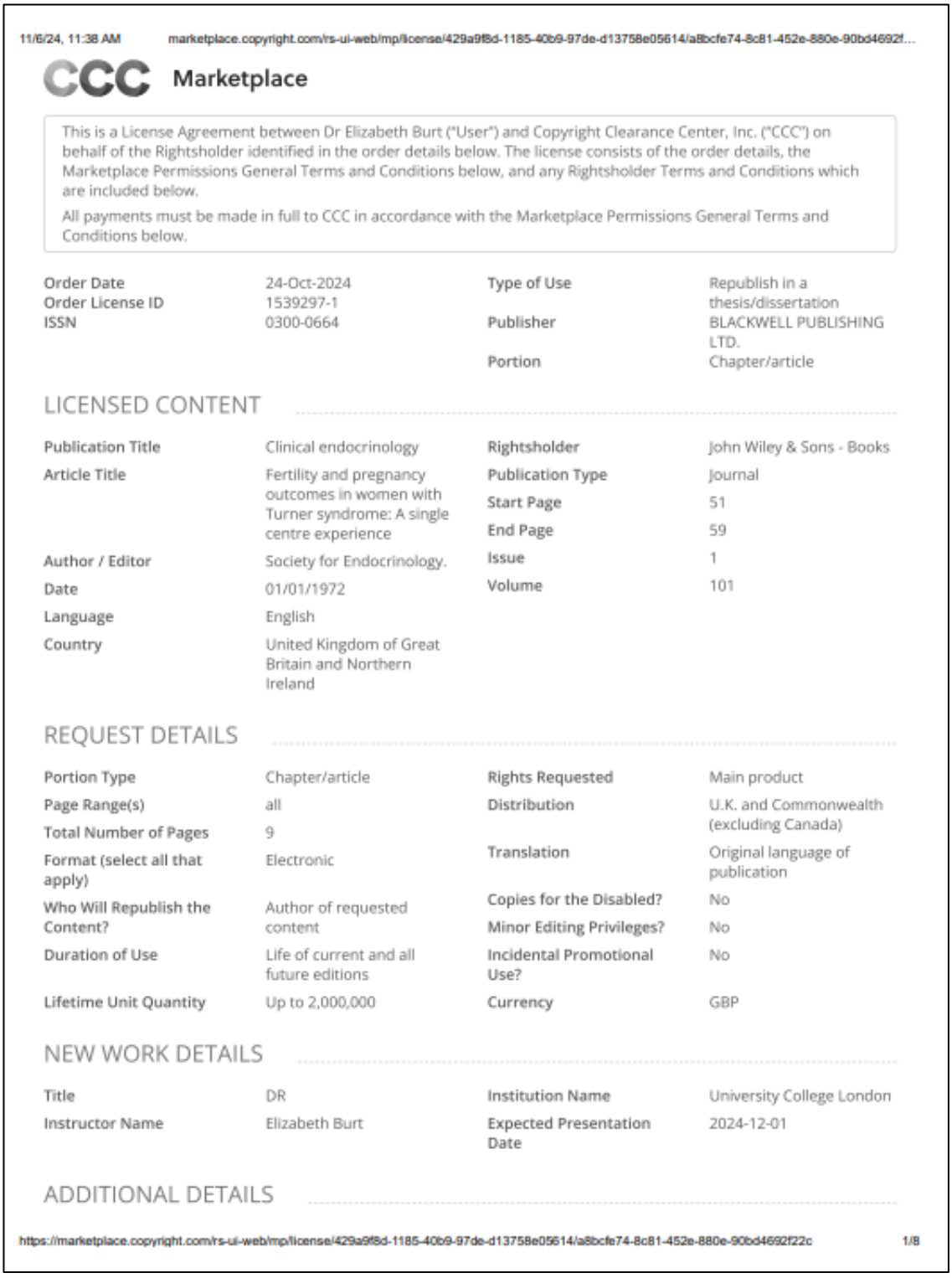

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APPENDIX 2. FINAL PUBLICATIONS

Fertility and Pregnancy Outcomes in Women with Turner Syndrome: A Single Centre Experience

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Fertility and pregnancy outcomes in women with Turner syndrome: A single centre experience

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Abstract

Objective: Many women with Turner syndrome (TS) will consider fertility options and pregnancy. We wished to examine the fertility and pregnancy outcomes in women with TS undergoing oocyte donation (OD) treatment or spontaneous pregnancy in a large single-centre cohort. General population reference data or data from those with idiopathic premature ovarian insufficiency were used as comparators.

Design: A retrospective single-centre cross-sectional study.

Patients and Measurements: Seventy-four women with TS underwent OD treatment with a total of 105 pregnancies, and 31 women with TS had 71 spontaneous conceptions. Fertility outcomes included clinical pregnancy and live birth rate. Pregnancy outcomes included miscarriage rate, prevalence of hypertension, gestational diabetes, lower segment caesarean section (LSCS), small for gestational age (SGA), prematurity and vertical transmission of TS.

Results: In those with TS, OD pregnancies were associated with increased rates of LSCS and SGA compared to spontaneous pregnancies; LSCS (OR: 4.19, 95% CI: 1.6–10.8, $p = .003$) and SGA (OR: 2.92, 95% CI: 1.02–8.38, $p = .04$). There were no recorded cardiac events but 5 (17.2%) cases of vertical transmissions of TS in daughters were identified. OD in those with TS was associated with a lower live birth rate per cycle started (OR: 0.53, 95% CI: 0.34–0.84, $p = .008$) and a higher rate of miscarriage compared to women with POI (40% vs. 26.2%, $p = .04$).

Conclusions: We show that pregnancy in women with TS, whether OD or spontaneously conceived, carries obstetric risks, and therefore, women with TS, considering pregnancy, should receive comprehensive pre-pregnancy counselling and optimal obstetric care.

KEYWORDS
oocyte donation, premature ovarian insufficiency, spontaneous pregnancy, Turner syndrome, uterus

1 | INTRODUCTION

Turner syndrome (TS), with partial or complete loss of the X chromosome, renders the ovarian germ cell pool vulnerable to hastened atresia.[1] Accelerated oocyte apoptosis and impaired folliculogenesis occur during the prenatal and early postnatal period, and the karyotype can be predictive for the degree of ovarian preservation and function.[2] Those with monosomy are most susceptible to complete oocyte depletion. However, a proportion of women with TS will retain some ovarian activity, with spontaneous menarche occurring in 5%–20% of women and spontaneous pregnancy reported in 2%–8%.[3,4] For those with ovarian insufficiency, oocyte donation (OD) IVF is a treatment option for conception.

Pregnancy in women with TS carries excess risks above the general population, irrespective of mode of conception, with increased miscarriage rates and maternal sequelae especially aortic dissection.[5–8] Furthermore, OD pregnancies in women without TS are associated with elevated risk of hypertensive disorders of pregnancy, preterm birth, low birth weight and increased rates of lower segment caesarean section (LSCS).[9–11] Women with TS who retain ovarian function and have a spontaneous pregnancy, still have increased obstetric risks including miscarriage[5,12–14] and also risk passing on chromosomal anomalies to their offspring.[12,14,15] The latter, however, is poorly quantified in the literature making accurate clinical counselling a challenge.

In this study, we set out to examine reproductive outcomes in women with TS from a large single-centre cohort. In particular, we sought to compare the maternal outcomes in our cohort who received regular health surveillance and cardiology review with previously reported literature. In addition, we were able to compare OD pregnancy outcomes in TS with those in women with other causes of premature ovarian insufficiency (POI) attending our centre. Previous literature has used comparative data from OD pregnancies with indications including increased maternal age and women with naturally declining ovarian reserve. Women with idiopathic POI provide a better comparison group, and there have been no direct comparisons of fertility and OD pregnancy outcomes between those with TS and POI.

2 | MATERIALS AND METHODS

2.1 | Participants

In this retrospective single-centre cross-sectional study, participants were recruited from the dedicated TS clinics of the Reproductive Medicine Unit at University College London Hospital (UCLH). These clinics have attendance from throughout the UK. However, fertility treatment and antenatal services usually took place at a hospital local to the participant's home. The inclusion criteria for recruitment were age above 16 years, a diagnosis of TS with OD or spontaneous pregnancy. TS was diagnosed clinically and confirmed with karyotype analysis.

2.2 | Clinical data

We sought to identify all women who had tried to conceive or who had been pregnant by individual interviews and detailed note review.

The TS karyotype was recorded and, for the purposes of analysis for outcomes, was categorised into 45,X or other. Age at each cycle of OD or at each pregnancy was collected in addition to their current age. Height, weight and BMI (kg/m^2) measurements were recorded before the first fertility treatment or first pregnancy. The presence of treated hypothyroidism before fertility treatment or pregnancy was recorded (excluding those who had subclinical hypothyroidism treated at the time of fertility treatment). Menstrual cycle history included a record of primary or secondary amenorrhoea or regular menstrual cycles. The presence of hypertension (HTN) or diabetes before fertility or pregnancy was also recorded.

The TS clinical care guidelines, published in 2017, provide guidance on pre-pregnancy care and echocardiogram assessment based on aortic size index (ASI) and cardiac pathology.[16] This level of care was already in place at UCLH before formalisation of the recommendations, having been developed over the preceding 20 years. Regular echocardiogram assessment for women with TS every 2–5 years is standard routine health surveillance at UCLH with additional pre-pregnancy assessment. Furthermore, a detailed plan for recommended antenatal monitoring, including cardiology surveillance, is provided once pregnancy is confirmed, as most women will receive antenatal care in their local area.

Cardiac status in TS included the presence of cardiac pathology (bicuspid aortic valve or coarctation and repair of the aorta). Echocardiogram assessment, ASI and cardiology assessment before fertility treatment or pregnancy were recorded.

2.3 | Fertility treatment

Fertility treatment occurred between the years of 1986–2023. Given the changes in practice over time, moving towards elective single embryo transfer (eSET), and the fact that multiple fertility centres with varying protocols contributed to this data, variables predicting outcome were restricted to the recipient characteristics. The age of the donor, endometrial preparation protocol and thickness, number and stage of embryos, the use of fresh or vitrified embryos and sperm source were not included in the analysis.

The primary outcomes analysed were pregnancy rate and live birth rate per cycle started, with the delivery of multiple pregnancy counted as one live birth event, which is consistent with the reporting of Human Fertilisation Embryo Authority (HFEA). Treatment cycles were categorised into first cycles and subsequent cycles.

2.4 | Pregnancy data

Pregnancies were recorded between 1979 and 2023. Pregnancy history included mode of conception, either spontaneous conception or with OD. Miscarriage was defined as a pregnancy loss before 23 weeks and 6 days gestation, not including termination of pregnancy or ectopic pregnancy. Live birth was defined as the delivery of a live baby (or babies) after 24 + 0 weeks gestation. Intrauterine death (IUD) was defined as foetal demise after 24 weeks of gestation. Termination of pregnancy and the reason, if known, was recorded.

Gravidity was the number of pregnancies, and parity was defined as the number of pregnancies that developed past 24 weeks gestation. For outcomes, pregnancy was categorised into the first pregnancy and subsequent pregnancies/deliveries.

Pregnancy complications were recorded for those that developed past 24 weeks and resulted in live birth or IUD. All pregnancies, including multiple pregnancies were recorded, however given the known increased incidence of adverse maternal and neonatal outcomes associated with multiple pregnancy, only singleton pregnancies were included in the analysis of pregnancy complications.[17,18] Hypertensive disorders of pregnancy consisted of either pregnancy-induced HTN and/or preeclampsia and excluded those who had preexisting HTN. The development of gestational diabetes mellitus (GDM) was recorded and excluded those with preexisting diabetes mellitus. Mode of delivery was categorised into vaginal delivery including both spontaneous and instrumental delivery and LSCS, both elective and emergency.

Neonatal outcomes for each singleton pregnancy resulting in live birth after 24 weeks gestation included gestational age (weeks) at the time of delivery, with preterm birth being defined as delivery less than 37 + 0 weeks' gestation.[19] Birthweight percentile was calculated[20] and small for gestational age was defined as a birthweight less than the 10th percentile. Information regarding the health of offspring was included and the presence of chromosomal anomalies was recorded.

2.5 | Comparison to normative data

Outcome data for TS spontaneous pregnancies were compared to normative general population data. The rate of miscarriage in the general population is 15.3%.[21] Normative data for singleton spontaneous pregnancies was adapted from Storgaard et al.[9] The percentages used in this meta-analysis of each original paper for spontaneous singleton pregnancies were used, and a mean was calculated.

2.6 | Comparison group with POI

Participants were identified from the dedicated POI clinics of the Reproductive Medicine Unit at UCLH. For the purposes of this study, POI was defined as hypergonadotrophic hypogonadism with karyotype 46XX. Subjects were excluded if POI had resulted from oncology treatment. Clinical data, fertility treatment and pregnancy data were recorded as above. For those with POI, only OD pregnancies after the diagnosis were included for analysis, although prior pregnancies were recorded for the purpose of defining gravidity and parity.

2.7 | Statistical analysis

Statistical analysis was completed using SPSS version 27 for Mac. The Shapiro–Wilk test was used to test the normality of continuous variables. Height, weight, BMI, age at time of first fertility treatment, number of fertility cycles, age at pregnancy, gravidity, parity, gestational age and birth weight percentiles were not normally distributed. Birth weight was normally distributed. Therefore, for uniformity, variables were described in frequencies and percentages or median, 5th and 95th percentile and nonparametric tests were used for analysis. χ^2 analysis was used to examine the differences in categorical variables and to compare TS spontaneous pregnancy outcomes to normative general population data.

For binary fertility and pregnancy outcomes, regression analysis with forward selection (likelihood ratio) was used. Adjustment was made for age and cycle number for fertility outcomes. For early pregnancy outcomes adjustment was made for age and gravidity. For maternal and neonatal outcomes, adjustment was made for age and parity, given the association between some adverse pregnancy outcomes and advanced maternal age and parity.[10,22,23] Results were presented as odds ratio (OR) and 95% CI. Statistical significance was defined as a p value less than .05.

3 | RESULTS

3.1 | Participants with TS

Seventy-four women underwent OD treatment, and 31 women had spontaneous conception. Three women had both OD treatment and spontaneous pregnancies. The clinical characteristics of each group are shown in Table 1.

Karyotype analysis was recorded in 93 (91.1%) women with TS. Of those undergoing OD treatment, 28 had monosomy X, 17 isochromosome X, 9 mosaic 45,X/46,XX, 6 mosaic 45,X/46,XY, 6 mosaic 45,X/46,XrX and 1 partial X deletion. Sixteen (21.6%) women had a cardiac pathology: 14 bicuspid aortic valves and two previous surgery for aortic coarctation. Eight (10.8%) women had preexisting HTN and 5 (6.8%) preexisting diabetes. Twenty-three (31.1%) women had been treated for hypothyroidism. Sixty (81.1%) had primary amenorrhoea, 13 (17.6%) had secondary amenorrhoea and 1 (1.4%) had a regular cycle.

Karyotypes in those with TS and spontaneous conception were 14 with mosaic 45,X/46,XX, 6 complex anomalies, 4 partial X deletion, 2 mosaic 45,X/46,XY, 2 mosaic 45,X/46,XrX, 1

TABLE 1 Participant characteristics in women with TS undergoing either OD treatment or with spontaneous pregnancy.

	Turner syndrome		
	TS OD treatment	TS spontaneous	p Value
Number of women	74	31	
Height (m)	1.50 (1.39–1.59)	1.50 (1.42–1.71)	.53
BMI (kg/m^2)	25.6 (20.0–36.6)	24.5 (20.3–37.5)	.46
Monosomy X	28/67 (41.8%)	1/30 (3.3%)	<.001
Cardiac pathology	16/74 (21.6%)	4/31 (12.9%)	.23
Hypothyroidism	23/74 31.1%	4/31 (12.9%)	.05
Primary amenorrhoea	60/74 (81.1%)	2/31 (6.5%)	<.001

Note: Results are displayed as either median and 5th–95th percentile or percentage. Note three women had both an OD treatment and a spontaneous pregnancy. *p* Values were displayed for the subgroup analysis between the two groups.

Abbreviations: OD, oocyte donation; TS, Turner syndrome.

isochromosome X and 1 monosomy X. Four (12.9%) had a cardiac pathology: 2 bicuspid aortic valve, 1 previous surgery for aortic coarctation and 1 valvular surgery. No women had existing HTN or diabetes. Four (12.9%) women had treated hypothyroidism. Two (6.5%) had primary amenorrhoea, 3 (9.7%) had secondary amenorrhoea, and 26 (83.9%) had a regular menstrual cycle.

Ninety-one (89.2%) women had an echocardiogram before pregnancy and 49 (48%) had a dedicated pre-pregnancy cardiology consultation. ASI results were available for 81 women (79.4%) as 8 women had an echocardiogram examination in their local hospital and two women had a cardiology assessment with echocardiogram and the aortic measurements were described as normal, but no ASI was reported.

ASI was greater than 2 cm/m^2 in 16/81 (19.7%) and 12 of these women had a specialist cardiology review also. No woman had an ASI > 2.5 cm/m^2.

3.2 | Pregnancy outcomes in TS spontaneous pregnancy

Thirty-one women with TS had 71 spontaneous conceptions. Pregnancy outcome data can be seen in Table 2. Forty- three pregnancies (60.6%) resulted in live birth and 45 children were born; 41 singletons and 2 sets of twins. Twenty-three pregnancies (32.4%) resulted in miscarriage. Five TOPs were recorded in 4 women, the reason being social in 3 women and non-disclosed in the fourth.

There was no case of aortic dissection. In singleton pregnancies, the incidence of HTN pathology and GDM was 12.5% and 15%, respectively. The mode of delivery was LSCS in 51.2% (13 elective and 8 emergency). The rate of preterm delivery was 2/38 5.3% and SGA was 6/38 15.8%.

There were 29 female (64.4%) and 16 male babies (35.6%) born to women with TS and spontaneous pregnancy, giving a M:F ratio of 0.55. which is significantly different than the expected 24:21 based on the ratio of 1.06 in the general population ($p = .01$).[24]

There were 5 cases (17.2%) of daughters born with TS. The associated maternal karyotypes were 2 with 46,X,t(X;Y)(p22.3;q11), 1 with 46X,del(X) (Xq21.3), 1 with 46,X,r(X) and 1 with 46X,del(X) (p21.1). In the first 3 cases, the daughter's karyotype was the same as the mother's and in the latter cases the daughter's karyotype was unknown. Two cases of vertical transmission occurred over three generations within the same family.

3.3 | TS spontaneous pregnancies vs population reference data

The incidence of miscarriage in women with TS and spontaneous pregnancy was higher than the miscarriage rate in all recognised pregnancies in the general population of 15.3%[21] ($p \leq .001$). Compared to population data of spontaneous singleton pregnancy, TS spontaneous singleton pregnancies were associated with an increased rate of HTN pathology (12.5% vs. 2.9%, $p \leq .001$), GDM (15% vs. 3.7%, $p \leq .001$), LSCS (51.2% vs. 16.9%, $p \leq .001$) and SGA (15.8% vs. 2.7%, $p \leq .001$). There was no significant difference in the rate of preterm delivery.

3.4 | OD fertility treatment in TS

Information regarding participants undergoing OD and outcomes can be seen in Table 3. Seventy-four women with TS underwent OD. Three women had a spontaneous pregnancy before embarking on fertility treatment.

Information was collected for 196 cycles of fertility treatment in women with TS. Four cycles did not result in embryo transfer and were abandoned. The median number of cycles per patient was 2 (5th–95th percentile 1–7). The clinical pregnancy rate and live birth rate per cycle started were 53.6% and 31.1%, respectively.

3.5 | Pregnancy outcomes in TS after OD

Information regarding pregnancy outcomes can be seen in Table 2. Sixty-five women with TS had a total of 105 pregnancies after OD. Sixty-one pregnancies (58.1%) resulted in live birth and 70 children were born; 53 singletons, 7 sets of twins and 1 set of triplets. Forty-two (40%) pregnancies ended in miscarriage and 2 (1.9%) resulted in ectopic pregnancy.

There was no case of aortic dissection during pregnancy in those with TS and OD pregnancy. In those with TS and OD singleton pregnancies, excluding those with preexisting HTN or diabetes, the incidence of HTN pathology was 8/48 (16.7%) and GDM 4/51 (7.8%). Caesarean section was the mode of delivery in 44/53 (83%)

TABLE 2 Pregnancy outcomes in women with TS undergoing either OD or spontaneous pregnancy compared to women with POI and OD pregnancy and general population data.

	TS OD	TS spontaneous	TS OD versus TS spontaneous	POI OD	TS OD versus POI OD	Normative data spontaneous singleton pregnancy
Women (*n*)	65	31		50		
Pregnancies (*n*)	105	71		84		
Number of children born	70	45		73		
Live birth rate	61/105 (58.1%)	43/71 (60.6%)	0.74	62/84 (73.8%)	0.02	
Miscarriage rate	42/105 (40%)	23/71 (32.4%)[a]	0.30	22/84 (26.2%)	0.04	15.3%
Termination of pregnancy	0	5/71 (7%)		0		
Intrauterine death	0	0		0		
Singletons	53/61 (86.9%)	41/43 (95.3%)		51/62 (82.3%)		
Twins (sets)	7/61 (11.5%)	2/43 (4.7%)		11 (17.7%)		
Triplets (sets)	1/61 (1.6%)	0		0		
Age at pregnancy	34 (26–43.4)	27 (18–39)	<0.001	34.5 (27–43.5)	0.14	
HTN pathology	8/48 (16.7%)	5/40 (12.5%)[b]	0.56	10/48 (20.8%)	0.60	2.9%
GDM	4/51 (7.8%)	6/40 (15%)[b]	0.27	8/48 (16.7%)	0.17	3.7%
LSCS	44/53 (83%)	21/39 (51.2%)[b]	0.005	28/49 (57.1%)	0.004	16.9%
Preterm birth	9/51 (17.6%)	2/38 (5.3%)	0.07	4/47 (8.5%)	0.18	4.7%
SGA	17/48 (35.4%)	6/38 (15.8%)[b]	0.04	13/47 (27.7%)	0.41	2.7%

Note: Results are displayed as either median and 5th–95th percentile or percentage. *p* Values displayed for the subgroup analysis of modes of conception in those with TS, between diagnostic groups or compared to general population data.

Abbreviations: GDM, gestational diabetes mellitus; HTN, hypertension; LSCS, lower segment caesarean section; OD, oocyte donation; SGA, small for gestational age; TS, Turner syndrome.

[a]Significantly different than general population data (all pregnancies).

[b]Significantly different from general population data (spontaneous singleton pregnancies).

(emergency LSCS in 17/44 38.6% and elective LSCS in 27/44 61.4%). The incidence of preterm birth and SGA was 17.6% and 35.4%, respectively.

3.6 | Comparison of the mode of conception in TS

Women with TS who had a spontaneous pregnancy were younger than those undergoing OD pregnancy. There was no difference in height or BMI between the two conception modes; however, those undergoing OD were more likely to have 45,X, and primary amenorrhoea compared to those with spontaneous pregnancies.

Compared to TS spontaneous pregnancies, the rate of LSCS was significantly higher in those with OD pregnancy (83% vs. 51.2%, $p = .005$) as was the rate of SGA (35.4% vs. 15.8%, $p = .04$). There was no difference in the rate of live birth, miscarriage, HTN, GDM or preterm delivery. Regression analysis adjusting for age at pregnancy and parity showed the raised rates of both LSCS (OR: 4.19, 95% CI: 1.61–10.8, $p = .003$) and SGA (OR: 2.92, 95% CI: 1.02–8.38, $p = .04$) remain significant with no other parameter reaching significance.

3.7 | Comparison of fertility and OD pregnancy outcomes between TS and POI

Fifty-three women with POI underwent OD (Table 3). Four had a spontaneous pregnancy before embarking on fertility treatment. There was no difference in the age of the first treatment cycle between TS and POI. Women with TS were significantly shorter and had a higher BMI compared to those with POI. Women with TS were significantly more likely to have primary amenorrhoea than those

TABLE 3 Fertility treatment outcomes in women with Turner syndrome or premature ovarian insufficiency undergoing oocyte donation treatment.

	Oocyte donation subgroup		
	TS	POI	p Value
Total number of women	74	53	
Height (m)	1.50 (1.39–1.59)	1.65 (1.51–1.77)	<.001
BMI (kg/m^2)	25.6 (20.0–36.6)	22.7 (18.9–34.9)	.008
Hypothyroidism	23/74 (31.1%)	11/53 (20.8%)	.19
Primary amenorrhoea	60/74 (81.1%)	11/53 (20.8%)	<.001
Number of cycles	196	136	
Number of women with prior pregnancy	3	4	
Number of cycles per woman	2 (1–7)	2 (1–5)	.63
Age at first treatment (years)	33 (25–43.5)	33 (25.7–43.3)	.78
Abandoned cycles	4/196 (2%)	5/136 (3.7%)	.36
The pregnancy rate per cycle started	105/196 (53.6%)	84/136 (61.8%)	.13
The live birth rate per cycle started	61/196 (31.1%)	62/136 (45.6%)	.007

Note: Results are displayed as either median and 5th–95th percentile or percentage. *p* Values displayed for the subgroup analysis across the different diagnostic groups.
Abbreviation: TS, Turner syndrome.

with POI. In those with POI, Fragile X premutation was identified in four women. The prevalence of treated hypothyroidism was not different between TS and POI. No women with POI had preexisting HTN or diabetes.

Information was collected for 136 treatment cycles in women with POI. Five cycles did not result in embryo transfer and were abandoned. The median number of cycles per patient was 2 (5th–95th percentile 1–5).

Whilst the pregnancy rate per cycle started was not different between those with POI or TS, the live birth rate per cycle started was lower in those with TS than POI (TS 31.1% vs. POI 45.6%, $p = .007$). Regression analysis demonstrated that when adjusted for age and cycle number, TS was associated with a lower live birth rate per cycle started (OR: 0.53, 95% CI: 0.34–0.84, $p = .008$) with no other parameter reaching significance.

In the POI group, 50 women had 84 OD pregnancies, and 73 children were born. The pregnancy outcomes can be seen in Table 2. In the OD group, those with TS, compared to POI, had lower live birth and increased miscarriage rate (live birth 58.1% vs. 73.8%, $p = .02$ and miscarriage 40% vs. 26.2%, $p = .04$) which both remained significant with regression analysis adjusting for age and gravidity; OR for live birth of 0.49 (95% CI: 0.26–0.91, $p = .02$) and an OR for miscarriage of 1.87 (95% CI: 1.00–3.50, $p = .04$) with no other factor reaching significance. The rate of LSCS was greater in those with TS pregnancy (83% vs. 57.1%, $p = .004$) and when adjusted for parity and age at pregnancy, the OR was 4.26 (95% CI: 1.63–11.1, $p = .003$). No difference between the diagnostic groups was identified in the rate of HTN pathology, GDM, preterm birth or SGA.

4 | DISCUSSION

In this paper, we set out to examine fertility and pregnancy outcomes in women with TS from a large single-centre cohort. We show that overall pregnancy in women with TS, whether OD or spontaneously conceived, carries obstetric risks above the general population. Reassuringly there were no recorded cardiac events in this cohort. In spontaneous pregnancies, five cases of vertical transmissions were identified. Women with TS who had OD had a higher rate of miscarriage compared to women with POI using OD.

Whilst TS OD pregnancy is associated with increased rates of LSCS delivery and SGA compared to TS spontaneous pregnancy, the risk for other outcomes with OD was not different from those in spontaneous pregnancies suggesting that there is not an excessive summative risk of TS with OD.

Cardiac health is a particular focus in women with TS considering a pregnancy. Interestingly, no cardiac event including aortic dissection occurred during the antenatal or post-partum period, which are events reported in the literature.[5–8] The 2017 published TS clinical care guidelines outline that an echocardiogram should be completed within 2 years before conception with calculation of the ascending ASI. If the ASI > 2.5 cm/m^2 or between 2.0 and 2.5 cm/m^2 with compounding cardiac pathology such as bicuspid aortic valve, coarctation of the aorta, HTN, elongation of the transverse aorta or history of aortic dissection then pregnancy should be avoided. During pregnancy, as aortic dissection has occurred in women with normal pre-pregnancy cardiac status,[6] an echocardiogram should occur at least once at approximately 20 weeks of gestation in the absence of

any identified risk factors or every 4–8 weeks during pregnancy and up to 6 months postpartum if other risks present.[16]

The more favourable cardiovascular outcomes detailed in this paper may reflect the early adoption of pre-pregnancy cardiovascular surveillance guidelines at UCLH. Cardiology assessment dates back to pregnancies in 2001 and an echocardiogram in 1999. In this TS cohort of women seeking fertility, the prevalence of cardiac pathology was lower than the cited rate in the overall TS population of 25%–50%[14,25] suggesting that a degree of case selection had taken place.

HTN during pregnancy is more prevalent in this TS cohort (both OD and spontaneous pregnancy) compared to spontaneous conception population data, however our paper did not find a difference in HTN between the modes of conception in TS, which is in contrast to previous papers suggesting a summative risk of OD and TS.[6,7,26,27] Other more recent papers have found comparable levels between the conception groups.[8,14] Reassuringly, whilst women with TS undergoing pregnancy still pose a higher cardiovascular risk, the rate of HTN pathology in those with TS and spontaneous pregnancy (12.5%) and those undergoing OD (16.7%) in our study was on the lower side of the other quoted rates in the literature 11%–20% in those with spontaneous conception[12,14] and 15%–62.5% for OD.[6,7,13,27–29] Furthermore, there was not a significant difference in the rate of HTN pathology between TS and POI OD pregnancies, which is in contrast to other papers.[26,27] There may be many variables affecting the prevalence of HTN, including local treatment thresholds and decision making by the local team.

Vertical transmission of TS is a known risk in women with spontaneous conception. This has been poorly documented in past literature and as systematic screening has not been undertaken the real incidence remains unknown.[12,14,15,30,31] In the study by Birkebaek et al., chromosomal analysis was completed for 25 of the 64 (38.4%) children born following spontaneous conception in those with TS. Five girls (5/32 15.6%) were found to have chromosomal aberrations consistent with TS.[32] Tarani et al. reported 13 spontaneous pregnancies in six women with TS, and out of eight live births, two daughters had TS.[31] Bernard et al. reported the outcomes from 56 spontaneous pregnancies in women with TS, and in those 30 children born, TS was detected in two daughters (6.6%) and trisomy 21 and 13 in others.[12] In the series by Calanchini, TS was diagnosed in one female in adulthood and she was found to have the same karyotype as her mother.[14]

We demonstrated a higher rate of vertical transmission than that previously highlighted in the literature partly because of one family passing on mosaic an X;Y translocation over three generations. This karyotype has been shown to be prone to transmission previously.[33] As a consequence of our findings, we recommend that preconception consultation should include a discussion regarding the option of prenatal or antenatal genetic counselling.

In those with TS undergoing OD, we found a pregnancy rate per cycle of 53.6% which is comparable to other papers of women with TS which vary between 33% and 57%.[27,29] Encouragingly, the live birth per cycle started in our cohort of 31.1% in those with TS is comparable to or higher than other reports of 3.2%, 17.9% and 27% and 33%.[6,14,27,32] We found that the pregnancy rate per cycle was similar between TS and POI, as has been reported by other groups comparing pregnancy rates to oocyte recipients for other indications.[32,34,35] We demonstrated a lower live birth rate per cycle started in women with TS undergoing OD compared to POI. This was not accounted for by abandoned cycles. One explanation may be deficient uterine and endometrial integrity in those with TS affecting optimal embryo implantation.

Miscarriage in TS is raised regardless of the mode of conception.[5,6,13,27,31,32,36] We identified a higher miscarriage rate in those with TS compared to both POI and general population data. In women with TS and OD pregnancies, the rates of miscarriage in the literature vary from 25% to 44% compared to our rate of 40%.[5,13,14,27,29] The higher miscarriage rate witnessed for both TS and POI with OD may be coupled with the higher prevalence of thyroid pathology and autoimmunity associated with these diagnoses.[16,37] Despite the occurrence of hypothyroidism being similar in those with TS and POI, there was still an elevated miscarriage rate and lower live birth rate in those with TS OD, suggesting that mechanisms other than thyroid dysfunction may be implicated, perhaps uterine factors. The earlier loss of ovarian activity in those with TS, compared to POI, leads to a higher proportion with primary amenorrhoea necessitating pubertal induction treatment. Despite seemingly sufficient exogenous oestrogen therapy, uterine size is often reduced compared to women with normal puberty.[38] However, the increased risk was also seen in those with secondary amenorrhoea and those with spontaneous conception, suggesting other compounding inherent factors affecting uterine function such as poor endometrial thickness, deficient uterine vascularity, collagen deficiency, lack of X-linked genes regulating endometrial receptivity or poor epithelial integrity with lack of tight junctions.[34,35,39,40]

The miscarriage rate in women with TS experiencing spontaneous pregnancy in this study was 32.4%, which falls in the ranges previously reported between 22.8% and 67.3%.[5,13,14,31] In our paper, we found a comparable rate of miscarriage in those with TS undergoing OD or spontaneous pregnancies which challenges the hypothesis previously suggested that oocytes from women the TS carry an increased risk of aneuploidy.[5,31]

The rate of LSCS was higher in women with TS irrespective of mode of conception and when compared with either population reference data or our POI cohort, consistent with previous reports.[6,11–13,34,39] The increased rate of LSCS in women has been linked to multiple factors, including short stature and feto-pelvic disproportion,[12,24] avoidance of exacerbation of underlying cardiovascular disease, expedited delivery due to maternal/foetal indications or patient preference. Whilst many women with TS will have an LSCS, and LSCS may be the preferred mode of delivery for some patients and obstetricians, it should not be overlooked that LSCS also poses risks and is itself associated with haemodynamic changes.[16] Therefore, LSCS should not be the default, and vaginal delivery should not be excluded. Notably, 29% of women with TS achieved a vaginal delivery, so a trial of labour may be possible depending on the multidisciplinary team review.

Pregnancies in women with TS have been shown to be associated with increased rates of prematurity and low birth weight.[6,7,9,12,13,27–29] This may be placentally driven[9] or alternatively, the HTN disorders may cause intrauterine growth restriction and, in turn, precipitate premature delivery. Concurring with this, we demonstrate higher rates of SGA in TS OD pregnancies compared to TS spontaneous conceptions and general population reference data. Premature delivery, however, was not found to be different between TS spontaneous and TS OD pregnancies, which is in contrast with some earlier studies,[7,27] but not others,[6,8] possibly reflecting differences in active management of complications minimising the need for expedited delivery and iatrogenic prematurity.

eSET should be standard best fertility practice and in those with TS planning OD treatment eSET should be particularly advocated to minimise the additional maternal and perinatal morbidity and mortality associated with multiple pregnancy.[17,18] The better maternofoetal outcomes in our paper maybe because we limited analysis to singleton pregnancies, however the multiple pregnancy rate in our TS OD cohort is low at 13.1%. Given our data covers several years predating the HFEA 'One at a Time' campaign in 2007, our rate of multiple pregnancies is encouraging, suggesting that patient education and adherence to policy is being met. Historically, eSET was less common, the paper by Chevalier et al. reported a double and triple embryo transfer of 40% and 18%, respectively[7] and multiple pregnancies may have contributed to increased adverse outcomes in earlier papers.

We demonstrated a predominance of female offspring following spontaneous conception in women with TS. This is to be expected as a 45,Y0 karyotype would not result in a viable pregnancy. Other authors have also commented on this finding with calculated M:F ratios between 0.37 and 0.84.[12,14,24]

We are aware of several limitations of our study. Information was gathered mainly by patient recall which may have led to some inaccuracies, however the reliability of self-reported pregnancy outcomes has been investigated by other groups, with reassuring results.[41] We were not able to obtain the karyotype of two of the daughters born with TS as the mothers were lost to follow-up. Locating all the antenatal cardiac data is challenging as antenatal care took place in other centres local to the patient's home. Furthermore, all karyotypes were not available as the paediatric data was not accessible in some cases.

In conclusion, women with TS considering pregnancy should receive comprehensive education regarding pregnancy and understand that overall pregnancy may pose a higher risk than those without TS. Pre-pregnancy counselling for those with TS who have the possibility of spontaneous pregnancy needs to address the possibility of vertical transmission. Overall our results on the risk of pregnancy are reassuring, and it is possible that improved cardiovascular screening and obstetric management are already showing the benefits of guidelines for adult care of women with TS.[16]

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Participants gave informed consent to take part in the study as part of the Turner Syndrome Life Project and The Reproductive Development Life Course Project (LO/2174 and 16/LO/0682 Chelsea Research Ethics Committee).

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Challenges in Developing a Quantitative Method of Measuring Breast Development Using 3D Imaging: An Example of a Novel Method for Use in Induced Breast Development with Exogenous Oestrogen

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DOI: 10.1111/cen.14815

ORIGINAL ARTICLE

WILEY

Challenges in developing a quantitative method of measuring breast development using 3D imaging: An example of a novel method for use in induced breast development with exogenous oestrogen

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Abstract

Objective: Optimal breast development is an essential part of exogenous oestrogen treatment in females undergoing pubertal induction. We set out to develop a novel technique using three-dimensional (3D) imaging to determine change in breast volume that is applicable when no pre-existing breast contours are present.

Design: A prospective observational study.

Patients: The imaging methodology was developed using a single male subject to assess reproducibility and validity. The technique was then applied to 29 participants undergoing pubertal induction with exogenous oestradiol who were recruited from Paediatric Gynaecology and Reproductive Endocrinology clinics at University College London Hospital.

Measurements: Breast images were taken using a 3D photographic system. Two images, taken at different times, were manually superimposed to produce a differential breast volume. The initial step of method development set out to show that volume change was not secondary to positioning artefact or image manipulation. This was established by using images of a male participant taken on different occasions. The technique was then used to assess reproducibility in participants undergoing pubertal induction treatment.

Results: Good intraobserver reproducibility (intraclass correlation (ICC) 0.77) was demonstrated with static image manipulation. Validity of the imaging technique was established as there was no significant difference between the known reference volume produced by computer generated warping and that calculated by manual image manipulation. There was excellent intraobserver reproducibility for breast volume calculation in participants undergoing induced breast development (ICC 0.99).

Conclusions: 3D imaging is a promising novel tool to provide quantitative breast volume assessment in individuals undergoing breast induction with exogenous oestradiol treatment.

KEYWORDS
breast, imaging, oestrogen, puberty, 3D

Variability of Response to Early Puberty Induction Demonstrated By Transverse Uterine Diameter Measurement and a Novel Method of 3D Breast Imaging

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ORIGINAL ARTICLE

WILEY

Variability of response to early puberty induction demonstrated by transverse uterine diameter measurement and a novel method of 3D breast imaging

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Abstract

Objective: Induction of puberty with exogenous oestrogen results in considerable variability in final uterine and breast volumes. We set out to quantify the variability of these two outcome measures with a view to establishing monitoring methods that could be used to individualise treatment protocols.

Design: A prospective observational study.

Participants: Sixteen participants with pubertal delay and primary amenorrhoea, due to hypogonadism were recruited from paediatric gynaecology and endocrinology clinics at University College London Hospital. A standardised protocol of transdermal 17β oestradiol (17βE) was used (Evorel™), with a starting dose of 12.5 mcg increasing to 25 mcg (patch changed twice weekly) after 4 months. Follow up was every 2 months for a total of 8 months.

Measurements: Uterine dimensions using ultrasound, oestradiol concentrations and breast development assessed by both Tanner staging and 3D photographic imaging.

Results: After 8 months of treatment, the changes in oestradiol concentrations (0–174 pmol), uterine volume growth (4.4–16.4 ml) and breast volume (1.76–140.1 ml) varied greatly between individuals. Of uterine parameters, transverse uterine diameter was most closely associated with serum oestradiol levels at 8 months (beta standardised coefficient = 0.80, $p = .001$). Change in breast volume was associated with age of treatment initiation (beta standardised coefficient 0.55 $p = .04$).

Conclusions: We demonstrate variation in response to exogenous oestrogen, emphasising the necessity for individualised dose titration. In the absence of sensitive oestradiol assays, uterine transverse measurements may be used as a surrogate marker of oestrogen sensitivity to guide early dose adjustment. 3D breast imaging may provide a quantitative assessment of breast development to complement Tanner breast staging.

Clinical Trials ID NCT02871986

Reduced Uterine Volume After Induction of Puberty in Women with Hypogonadism

Received: 6 March 2019 | Revised: 1 September 2019 | Accepted: 2 September 2019
DOI: 10.1111/cen.14092

ORIGINAL ARTICLE

WILEY

Reduced uterine volume after induction of puberty in women with hypogonadism

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Abstract

Objective: Adequate uterine growth is an essential component of pubertal induction with exogenous oestradiol in those with hypogonadism. Poor uterine development will render the individual vulnerable in the context of fertility. We assessed uterine size using ultrasound in those who had undergone pubertal induction treatment compared with a reference group who had experienced spontaneous puberty.

Design: This is a single-centre, retrospective, cross-sectional study of women who underwent pubertal induction compared with a reference group.

Patients: Ninety-five women with hypogonadism who had previously undergone pubertal induction and were receiving maintenance oestrogen replacement as adults were recruited: 48 women with Turner syndrome, 32 with premature ovarian insufficiency and 15 with gonadotrophin deficiency. The reference group consisted of 35 nulliparous women attending with male factor subfertility with a normal pelvis on ultrasonography.

Measurements: Pelvic ultrasound was performed by a single observer. Uterine dimensions (total length, anterior–posterior (AP), transverse, uterine volume and fundal cervical AP ratio (FCR) measurements) were recorded. Clinical details were also recorded.

Results: Those with hypogonadism had significantly reduced uterine dimensions compared with the reference group (uterine length 64 mm vs 71 mm $P = <.05$, uterine volume 28.9 mL vs 43.9 mL $P = <.05$). All women in the reference group attained a mature uterine configuration with a FCR >1, compared with 84% of those with hypogonadism ($P = .01$). A total of 24% and 48% of the diagnostic group had total uterine length and uterine volume measurements less than the 5th percentile of the reference group, respectively. In a subgroup of 22 women in whom serum oestradiol concentrations could be analysed, there was a positive correlation between this parameter and uterine volume.

Conclusion: Despite standard oestrogen therapy, uterine growth is often compromised in those with hypogonadism. Uterine health has historically been overlooked in pubertal induction protocols; however, with increasing options for fertility treatment, adequate uterine development is crucial. Given the variation in uterine size witnessed, a more tailored approach to treatment with regular monitoring of uterine dimensions should be advocated.

APPENDIX 3. STUDY DOCUMENTS FOR ETHICS APPLICATION

STUDY PROTOCOL

University College London Hospitals NHS
NHS Foundation Trust

VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM

Protocol Version: 1.0

Protocol Date: 15th September 2016

Chief Investigator:

Professor Gerard Conway, MD, Consultant Endocrinologist

Student Investigator & Principal Investigator:

Dr Elizabeth Burt MRCOG, Clinical/Research Fellow

Sponsored by:

University College London (UCL)

Protocol Version and Date:

1.0

15th September 2016

IRAS Project ID:

199997

Study Registration Number:

R&D 16/0332

PROTOCOL VERSION

Version Stage	Versions No	Version Date	Protocol updated & finalised by;	Appendix No detail the reason(s) for the protocol update
Current	1.0	15/09/2016	Prof Conway	

Declarations

The undersigned confirm that the following protocol has been agreed and accepted and that the investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the Research Governance Framework 2005 (as amended thereafter), the Trust Data & Information policy, Sponsor and other relevant SOPs and applicable Trust policies and legal frameworks.

I (investigator) agree to ensure that the confidential information contained in this document will not be used for any other purposes other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I (investigator) also confirm that an honest accurate and transparent account of the study will be given; and that any deviations from the study as planned in this protocol will be explained and reported accordingly.

Chief Investigator:

Signature:..... Date...../...../.....

Print Name (in full):.....

Position:.....

On behalf of the Study Sponsor:

Signature:..... Date...../...../.....

Print Name (in full):.....

Position:.....

STUDY SUMMARY

Identifiers	
IRAS Number	199997
REC Reference No	R&D 16/0332
Sponsor Reference No	N/A
Other research reference number(s) (if applicable)	Puberty Induction - Ref 07/H0716/63 Reproductive Life Course Project- Ref 16/LO/0682
Full (Scientific) title	Variability of uterine and breast development in response to exogenous oestrogen during induction of puberty in individuals with hypogonadism.
Health condition(s) or problem(s) studied	Idiopathic Hypogonadotrophic Hypogonadism, Hypothalamic Amenorrhoea, Hypopituitarism, Primary Ovarian Insufficiency, Turner Syndrome, Transgender.
Study Type i.e. Cohort etc.	Prospective observational study
Target sample size	24
STUDY TIMELINES	
Study Duration/length	4 years
Expected Start Date	01/10/2016
End of Study definition and anticipated date	01/10/2020
FUNDING & Other	
Funding	No funding required for this study
Other support	No support required
STORAGE of SAMPLES **(if applicable)**	
Human blood samples	UCLH approved freezers
Data collected / Storage	Password protected UCLH computers, UCLH approved encrypted USB and hard drive
KEY STUDY CONTACTS	Full contact details including phone, email and fax numbers
Chief Investigator	Prof. Gerard Conway, gerard.conway@uclh.nhs.uk, 020 3456 7890 75701.

KEY ROLES AND RESPONSIBILITIES

SPONSOR: The sponsor is responsible for ensuring before a study begins that arrangements are in place for the research team to access resources and support to deliver the research as proposed and allocate responsibilities for the management, monitoring and reporting of the research. The Sponsor also has to be satisfied there is agreement on appropriate arrangements to record, report and review significant developments as the research proceeds, and approve any modifications to the design.

FUNDER: The funder is the entity that will provide the funds (financial support) for the conduction of the study. Funders are expected to provide assistance to any enquiry, audit or investigation related to the funded work.

CHIEF INVESTIGATOR (CI): The person who takes overall responsibility for the design, conduct and reporting of a study. If the study involves researchers at more than once site, the CI takes on the primary responsibility whether or not he/she is an investigator at any particular site.

The CI role is to complete and to ensure that all relevant regulatory approvals are in place before the study begins. Ensure arrangements are in place for good study conduct, robust monitoring and reporting, including prompt reporting of incidents, this includes putting in place adequate training for study staff to conduct the study as per the protocol and relevant standards.

The Chief Investigator is responsible for submission of annual reports as required. The Chief Investigator will notify the RE of the end of the study, including the reasons for the premature termination. Within one year after the end of study, the Chief Investigator will submit a final report with the results, including any publications/abstracts to the REC.

PRINCIPLE INVESTIGATOR (PI): Individually or as leader of the researchers at a site; ensuring that the study is conducted as per the approved study protocol, and report/notify the relevant parties – this includes the CI of any breaches or incidents related to the study.

KEY WORDS

Puberty, Puberty Induction, Primary Amenorrhoea, Hypogonadism, Uterus

LIST OF ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
PI	Principle Investigator
PIS	Participant Information Sheet
REC	Research Ethics committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
POI	Primary Ovarian Insufficiency
PCOS	Polycystic Ovarian Syndrome
HA	Hypothalamic Amenorrhoea
HP	Hypopituitarism
HH	Hypogonadotrophic hypogonadism

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INTRODUCTION & BACKGROUND

Working Title:

Variability of uterine and breast development in response to exogenous oestrogen during induction of puberty in Individuals with hypogonadism.

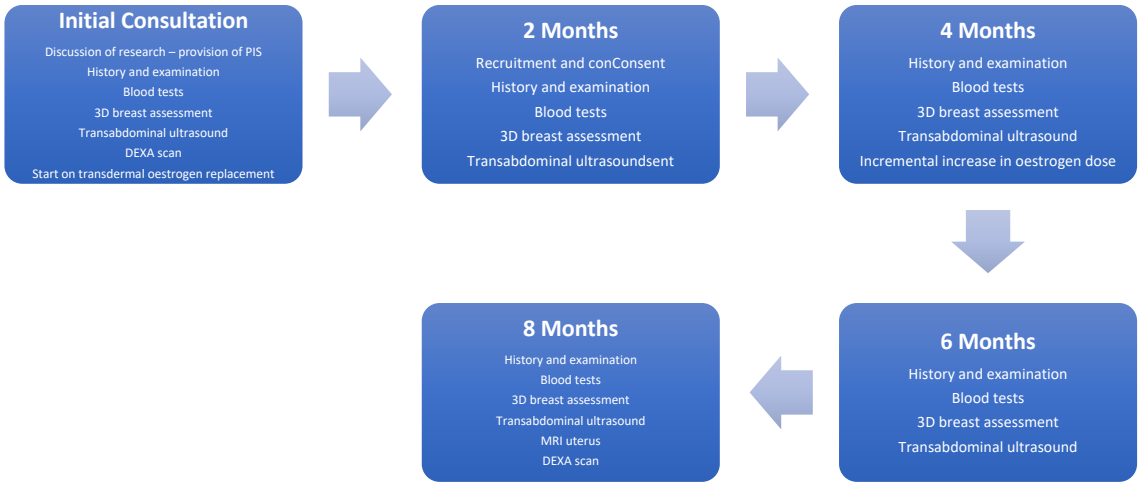
Research Subject Area:

Oestrogen replacement in individuals with hypogonadism

Introduction:

Oestradiol is required for the mature uterine configuration and breast growth. Historic data is poorly documented and there is divergence with regards to pubertal induction protocols. Therefore we need improved precision in pubertal induction management. The aims of the study are to further understand pubertal induction by observing individuals with hypogonadism, undergoing pubertal induction and auditing outcome measures including uterine, breast and bone development.

Study Flowchart:



Background:

Oestrogen deficiency is manifest in three ways depending on the stage in life when it occurs - delayed puberty, secondary amenorrhoea and menopause.

Puberty is dependent on an appropriately functioning hypothalamic- pituitary – ovarian axis culminating in the development of secondary sexual characteristics.

Puberty is a slow linear process taking several years for completion. The average age of thelarche (breast development Tanner Stage ≥ B2) is 11.3 years, with menarche occurring approximately 2 years thereafter. Lack of thelarche by the age of 13 or primary amenorrhoea after the age of 15 is indicative of oestrogen deficiency. This is turn may be secondary to a spectrum of pathologies.

Induction of puberty aims to mimic normal physiology of spontaneous puberty over several years, with the use of exogenous oestrogen in individuals who are hypogonadotrophic. There is no consensus with regards to protocols for oestrogen replacement in puberty induction. Treatment needs to be tailored to each individual depending on underlying cause and time of presentation. In UK practice, oral ethinylestradiol (EE) or transdermal (TD) patches/gel 17β-oestradiol are favoured over conjugated equine oestrogen (CEE) preparations or the combined oral contraceptive pill (COCP). Neither EE or TD preparations undergo first pass hepatic metabolism and therefore can be used in lower doses. CEE use in children is not recommended as it also

contains non-human androgens and progestogens, whilst COCP contains too high levels of synthetic oestrogen and progestogen, which may have a negative impact on breast and uterine development.

The timing of first exposure to oestrogen is controversial, as the need for oestrogen replacement to attain adult sexual development needs to be carefully balanced with the necessity for optimal long bone growth. Excessive early oestrogen may precipitate premature epiphyseal closure with a negative effect on adult height. However a delay in starting puberty induction may have adverse consequences on peak bone accrual and cognitive function and in addition may result in negative body image and psychological effects.

To circumvent these concerns and produce a more accurate replication of spontaneous puberty new strategies have been advocated. GH, if required, can be started at a younger age with oestrogen in smaller doses following at approximately 12 years of age. Oestrogen dosage can be then titrated up accordingly. In contrast to traditionally larger doses of oestradiol, this treatment strategy using earlier more conservative oestradiol dosing, may not compromise final height and may convey more beneficial effects on breast and uterine development, bone mineralization and memory.

Ultimately the aims for puberty induction are multifactorial, based on several outcome measures. Several endpoints may be used to monitor the pace of artificial puberty including uterine dimensions, body shape, breast size/volume and staging.

Transabdominal scanning is a minimally invasive imaging modality, commonly used within the sphere of gynaecology. With uterine growth being oestrogen dependent uterine dimensions can provide vital information to assess pubertal status, and aid both clinical diagnosis and management. To allow clinical integrity and applicability, reproducibility of the technique is essential. More recently ultrasound advances with 3D software allows alternative volume assessment and MRI has become a complementary diagnostic tool for evaluating uterine and ovarian parameters.

Uterine growth begins prior to thelarche and it has been postulated that prepubertal oestradiol levels have an influence on final uterine growth potential. Uterine development begins at approximately 7-8 years of age, continues throughout the pubertal years and endures beyond that of menarche and full breast development. Uterine development ceases at approximately 20 years of age. Ovarian growth commences approximately 2 years after the start of uterine growth. Uterine growth may be compromised secondary to oestradiol deficiency during this time, resulting in a hypoplastic uterus.

Thelarche is the development of a mature and functional mammary gland composed of adipose, lobules and duct cells with the external appearances of the nipple and areola. Full breast development takes on average 4 years. Although some in utero and neonatal development of rudimentary breast tissue does occur in a similar fashion to the uterus and ovaries, again the vast majority of development is oestrogen dependent and occurs during the pubertal years. Mammogenesis is dependent on not only oestradiol but also, similarly to the reproductive organs and bone; GH and Insulin Like Growth Factor 1 (IGF-1) are thought to act as adjuvants to proliferation, with IGF-1 and oestrogen acting in a synergistic fashion.

Classification and clinical assessment of breast development can be standardised using Tanner Stages, first described in 1962. This is routinely used in clinical practice however is limited by requiring subjective judgement. Other modalities for breast imaging/assessment used in other disciplines such as Plastic surgery and Oncology include MRI, ultrasound and 3D laser scanning.

Much of our knowledge on induction of puberty and uterine and breast development has been derived from the study of females with Turner Syndrome (TS), with diverse outcomes. Some studies have suggested that adult uterine shape can be attained with exogenous oestrogen treatment, whilst others have reported to the contrary, with poor uterine and breast development despite replacement.

In summary, there are disparate conclusions drawn and the published evidence is concentrated on the TS population. Greater exploration into the concept of oestrogen replacement in those with other causes of hypogonadism will allow more understanding into the natural history of oestrogen

deficiency on uterine and breast development. The aim is to provide more bespoke and evidenced based therapeutic interventions.

OBJECTIVES

Primary Objectives:

- Variability of uterine dimensions as assessed by a single observer using transabdominal ultrasound (TAUS) at baseline and then at 2 monthly intervals for a total of 8 months will be compared to serum oestradiol concentrations.
- Variability of breast dimensions and volume using Tanner staging and 3D breast imaging

Secondary Objectives:

- Anthropometric measurement – height, weight, BMI, BP, body fat measurement, hip measurement, waist measurement
- Bone – osteocalcin, bone ALP, DEXA
- Uterine assessment by MRI
- Hormonal profile – FSH, LH, sensitive oestradiol levels, IGF-1, TFT, FT4
- Tanner stage of pubic and axillary hair
- Inter-observer and intra-observer agreement of ultrasound measurement

Associated Ethics:

1. Puberty induction - Ref 07/H0716/63
2. The Reproductive Development Life Course Project – Ref 16/LO/0682

STUDY DESIGN & METHODS

Design Summary:

This will be a prospective observational study

Study Population:

Individuals with hypogonadism who require pubertal induction

Expected Duration

The total time for each participant in the study will be a total of 8 months. They will be assessed every 2 months for a total of 8 months and then followed up in the standard manner.

Number of Participants:

This has been based on the knowledge of the size of the available clinical cohort

STUDY SCHEDULE:

Subject Screening & Enrolment:

Individuals who have been referred with a history of hypogonadism will attend specialised clinics at UCLH. A member of the research team will screen the GP referral letter and specific clinic lists to identify potential participants.

All individuals attending the Endocrinology, Women's Health Clinics and Paediatric Endocrinology Clinics at UCLH, with a diagnosis of hypogonadism will be considered eligible for the study.

Subject Recruitment:

Individuals with a history of hypogonadism will be referred and will attend a clinic appointment in the standard manner. A member of the research team (also part of the direct care team) will screen patient for eligibility at the initial consultation.

The potential participant and, if the participant is <16 age, the person with parental responsibility will be approached by one of the members of the research team.

The researcher will describe the research and provide the participant +/- the person with parental responsibility with a PIS detailing the outline of the study. The participants +/- person with parental responsibility who agree to take part in the study will be asked to sign a consent form, which will be filed in a secure location.

For the participants under the age of 16 two dedicated consent forms will be used for the participant and the person with parental responsibility.

Participant Withdrawal:

Participants will be free to withdraw consent at any point during the study. Their information will be removed, and they will continue to be followed up in the standard manner. This will not affect their care in any way.

End of Study:

The end of the study will be when the last participant has been recruited and finished 8 months of follow up. There will then be a period of data analysis.

CONSENT

Consent will be taken by one of the research team who has dedicated consent training. The consent process will depend on the age of the participant:

<16 years of age – consent gained from both patient and person with parental responsibility (assent form for participant and consent form for person with parental responsibility)

≥ 16 years of age – consent gained from participant.

The PIS and consent form will be age specific. For those aged less that 16 there will be dedicated PIS for both the participant and the person with responsibility. A consent form and an assent form will also be used. For those age over 16 and over there will be both a consent form and a PIS.

For those with limited English a translational service e.g. language line will be used

ELIGIBILITY CRITERIA

Inclusion criteria:

- Diagnosis of hypogonadism (Turner's syndrome, hypogonadotrophic hypogonadism, Premature Ovarian Insufficiency, hypopituitarism, hypothalamic amenorrhoea, transgender individuals)
- Individuals ≥ 10 years of age
- Oestrogen naïve i.e. no prior commencement of treatment
- Breast Tanner stage ≤ than 2

Exclusion Criteria:

- Previous oncology treatment
- Primary amenorrhoea secondary to chronic medical illness
- PCOS diagnosis
- < 10 years of age
- > 30 years of age

INITIAL ASSESSMENT & FOLLOW UP

HISTORY AND EXAMINATION:

The researcher will obtain a full medical history:

- Diagnosis:
- Medical co-morbidities
- Medications
- Allergies
- Family history

ANTHROPOMETRIC MEASUREMENTS

Height: Measured using stadiometer to the nearest mm with. The measurement will be taken with the subject's feet together and the head in a horizontal plane. The heels, buttocks and shoulder blades should be against the stadiometer.

Weight: Measured to the nearest 0.1kg using electronic scales

Body Mass Index (BMI): kg/m^2

Blood Pressure: mmHg using a suitable sized cuff

Hip: Waist Measurement and Ratio: Waist circumference will be measured in cm to the neatest mm, between the lowest border of the rib cage and the upper border of the iliac crest. Both landmarks should be marked, and the midpoint should be determined. The measurement should be taken with the participant standing and at the end of a normal expiration. Hip circumference is again measured in cm to the nearest mm at the level of the maximum buttock projection with only underwear on. Measurement will be taken using inextensible tape, which will be placed tightly, but not compressing the tissues.

Body Fat Measurement: Tanita machine will be used as per standard settings.

BREAST AND PUBERTAL ASSESSMENT:

Tanner Stage: Assessment according to Tanner staging for breast, axillary and pubic hair

3d Surface Imaging System: 3d breast imaging using 3dMD photogrammetric system. This will allow assessment breast morphology +/- volume. Subjects will be in a seated position. The image taken will be from the sternal notch to inferior rib. No face/head image will be included. Defined landmarks will be placed on the participant's torso using removable marker (individual use for each patient) and removed after the image has been obtained.

BLOOD TESTS:

The researcher for will take blood tests at initial consultation:

- Follicle Stimulating Hormone (FSH)
- Luteinizing Hormone (LH)
- Oestradiol - Roche Emodular assay UCLH
- Anti-Mullerian Hormone (AMH)
- Insulin -Like Growth Factor -1 (IGF-1)
- Thyroid Stimulating Hormone (TSH)
- Free T4 (FT4)

These are non-fasting samples and will be taken in gold top biochemistry bottles. These form the standard routine assessment bloods. Samples will be processed at the biochemistry laboratory at University College London Hospital in the standard manner. Oestradiol measurements at the UCLH biochemistry are completed using an immunoassay Roche Emodular assay with a lower detection limit of 44pmol/L

Blood tests will be also taken for

- Sensitive oestradiol by LC-MS/MS (Liquid chromatography tandem mass spectrometry)
- Sensitive estrone by LC-MS/MS
- Osteocalcin
- Bone ALP

Blood will be taken in gold top bottles. The blood will be centrifuged to obtain serum in a dedicated research laboratory, which will be then stored in vials in compliant laboratory freezers (as per laboratory instruction). Stored samples will be held within the reproductive medicine laboratory, UCLH and will be labelled with only study number. The samples may be kept for up to 5 years after the end of the study period for further analysis, subject to further ethics application.

Blood vials will be couriered (as per laboratory instruction) to South Manchester Hospital (Wythenshawe) for further assessment by liquid chromatography tandem mass spectrometry (sensitive oestradiol and estrone) or TDL laboratory (Bone ALP and osteocalcin)

At each visit, all blood tests can be taken with one needle stick.

ULTRASOUND:

A baseline gynaecology ultrasound scan will be requested as part of standard assessment. The principle investigator of the study will complete the ultrasound. The ultrasound examination will follow a standard protocol for trans-abdominal scanning.

The data will be recorded on a clinical/ultrasound database (Viewpoint ultrasound database, version 5.6.8.428, Bilderararbeitung, GmbH).

INTRA & INTER OBSERVER RELIABILITY OF ULTRASOUND:

On one occasion, a second member of the research team will repeat the scan. The second investigator will enter the room and will be both blinded to the clinical history and the principal investigators measurements. The same settings of the ultrasound machine will be used. They will compete one set of measurements.

Alternatively, the 3d image will be taken 3 times and the most appropriate image will be stored on the UCLH ultrasound machines and patient identifiable information will be removed. Another member of the research team will manipulate the image to obtain the standardised views and measurements. These will be recorded. The initial investigator will also repeat the measurements.

MRI:

An MRI scan will be completed after 8 months of treatment and uterine size and volume will be assessed. Standard MRI settings will be used for all patients.

BONE DENSITY:

A dual emission x-ray absorptiometry (DEXA) of hip and L1-L4 spine will be performed as part of the standard assessment to be conducted at the University College London Hospital.

ROUTINE INTERVENTION

Transdermal 17β oestradiol (17βE) will be prescribed (Evorel™). Evorel is a square shaped transparent self-adhesive transdermal delivery system with a thickness of 0.2mm. It consists of a monolayered matrix throughout which the 17βE is uniformly distributed.

Evorel 25 has a surface area of 8 sq. cm and contains 1.6mg oestradiol, corresponding to a release rate of 25mcg per 24 hours

Treatment will commence, with a dose:

- 12.5 mcg/24h transdermal 17βE for 4 months (to be changed twice weekly). A 25mcg patch will be cut in half in order to obtain the dose of 12.5mcg/24h. After 4 months the dose will increase to 25 mcg/24h (to be changed twice weekly).

Patch change: will be on Thursday and Sunday

FOLLOW UP

Participants will be followed every 2 months on a Monday or Friday.

At each 2-month appointment the following will be repeated:

- Anthropometric measurements
- Blood tests for FSH, LH, Oestradiol (Roche), Oestradiol and estrone sensitive, and AMH
- +/- Ultrasound will be repeated

After 8 months:

- A repeat DEXA scan
- A repeat blood tests for TSH, FT4, IGF-1, osteocalcin and bone ALP
- +/- MRI

STATISTICAL ANALYSIS

Sample size of participants was based on an estimation of the current clinic size for each of the conditions. Statistical methods of analysis may include but not be exclusive to ANOVA, Chi-squared distribution and bivariate analysis. Baseline data that will be collected is outline above. Data will be analysed using SPSS statistical software version 22.0 for Mac

PATIENT AND PUBLIC INVOLVEMENT

There has been no patient and public involvement.

FUNDING AND SUPPLY OF EQUIPMENT

NHS costs will be supported via UCLH. Any additional costs such as the additional oestradiol assays will be covered by the research budget of Professor Conway.

DATA HANDLING & MANAGEMENT

Data collection will occur on a data collection sheets. A member of the research team/direct care team will collect this data. Data will be also recorded, as per the standard manner, in the participant's main medical notes as this study represents the observation and auditing, of routine clinical care.

Data sheets will only include study number and no other patient identifiers. Hard copies of the data sheets will be held in a locked cupboard/ locker within the Reproductive Medicine Unit (UCLH).

Completed consent forms will also be held in a locked cupboard/locker with the Reproductive Medicine Unit (UCLH).

The consent forms and the data collection sheets will be held separately.

A secure spread sheet will be used to link the data, and this will be kept on password protected UCLH /UCL computers and will only be accessed by members of the research team.

Ultrasound and MRI images will be held on the appropriate secure NHS imaging software on UCLH computers as these form part of the routine medical records for the patient.

Breast images will be held on secure NHS UCLH secure computers.

Blood samples to be stored will be coded according to hospital number and visit number, which is non-identifiable outside of UCLH, as a means of identification. Only members of the research team will have access to these samples. Samples that are required to be couriered to another laboratory will be done so using an NHS approved courier service.

Routine blood tests will be processed and stored as per UCLH laboratory protocol.

Elizabeth Burt or Prof, Gerard Conway, 250 Euston Road, 2nd Floor North, Women's Health, NW1 2PG UCLH will process, store and dispose of patient data in accordance with all applicable legal and regulatory requirements, including the Data Protection Act 1998 and any amendments thereto.

Confidentiality and Security:

All patient records will be handled according to NHS confidentiality practices. Samples will be coded and sequentially numbered. The principal investigator will handle patient identifiable data. All electronic data will be kept securely on NHS password protected computers or UCLH encrypted removable electronic media. Patient identifiable data will not leave NHS hospital premises.

MATERIAL/SAMPLE STORAGE

In the study, blood samples will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them. Samples will be processed, stored and disposed in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereafter.

Samples to be sent to the main UCLH laboratory will be labelled with name, hospital number and DOB in accordance with hospital policy. Stored samples will be coded according to study number and visit code. Stored samples will be held in approved laboratory freezers. Those to be couriered will be done do in accordance with laboratory protocol.

PEER AND REGULATORY REVIEW

The study has been peer reviewed in accordance with the requirements outlined by UCL

Having discussed with the UCL Research Office:

- This study has been reviewed as part of an educational programme. The Sponsor has verified that the supervisor of the project has undertaken sufficient review of the protocol in line with the requirements of his/her department.

STUDY ADMINISTRATION

Conflicts of Interest:

Nil

Subject Stipends or Payments:

There will be no stipends or payments.

Study Timetable:

The study will recruit over 1.5 years. Follow up will be a total of 8 months.

ASSESMENT AND MANAGEMENT OF RISK

The study will review routine management of pubertal induction. Therefore there should be minimal additional risks as consequence of this study. Examination, ultrasound, blood tests and DEXA scans are common tools to assess pubertal status during and after completion of puberty induction. A transabdominal ultrasound scan will be performed in the standard manner. If additional incidental pathology is identified as part of the routine assessment, then the patient will be referred or receive follow up in according to local protocols. An MRI is an alternative modality used to assess uterine measurements. It is a non-invasive test and not associated with ionizing radiation.

3D breast imaging is another non-invasive and quick method of assessing breast status. Images will not include the head or face. Images will be stored and coded by study number and all identifiable information will be removed.

The process of pubertal induction may lead to psychological concerns for the patients. The clinical team at UCLH compromised doctors, psychologists and clinical nurses all of whom are equipped to counsel subjects if required.

Of upmost importance will be to keep and protect patient data in accordance with the Data Protection Act and policies implemented by UCLH. All clinical data will be held on secure NHS computers within the Department of Women's Health UCLH, UCL computers and trust approved encrypted USB and hard drive devices.

RECORDING AND REPORTING OF EVENTS AND INCIDENTS

Definitions of Adverse Events:

Term	**Definition**
Adverse Event (AE)	Any untoward medical occurrence in a patient or study participant, which does not necessarily have a causal relationship with the procedure involved.
Serious Adverse Event (SAE).	Any adverse event that: • Results in death, • Is life-threatening*, • Requires hospitalisation or prolongation of existing hospitalisation**, • Results in persistent or significant disability or incapacity, or • Consists of a congenital anomaly or birth defect
*A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe. ** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE.	

Assessments of Adverse Events:

Each adverse event will be assessed for severity, causality, seriousness and expectedness as described below**.**

Severity:

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require further procedure; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the participant's routine, or requires further procedure, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

Causality:

The assessment of relationship of adverse events to the procedure is a clinical decision based on all available information at the time of the completion of the case report form.

The differentiated causality assessments will be captured in the study specific SAE form

The following categories will be used to define the causality of the adverse event:

Category	Definition
Definitely:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Possibly	There is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the study procedure). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant events).
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the study procedure). There is another reasonable explanation for the event (e.g. the participant's clinical condition).
Not related	There is no evidence of any causal relationship.
Not Assessable	Unable to assess on information available.

Expectedness:

Category	Definition
Expected	An adverse event which is consistent with the information about the procedure listed in the Investigator Brochure, SPC, manual of Operation
Unexpected	An adverse event which is not consistent with the information about the procedure listed in the manual of operation

Recording Adverse Events:

All adverse events will be recorded in the medical records in the first instance.

All adverse events will be recorded with clinical symptoms and accompanied with a simple, brief description of the event, including dates as appropriate.

Procedures for Recording and Reporting Serious Adverse Events:

All serious adverse events will be recorded in the medical records and the CRF, and the sponsor's AE log

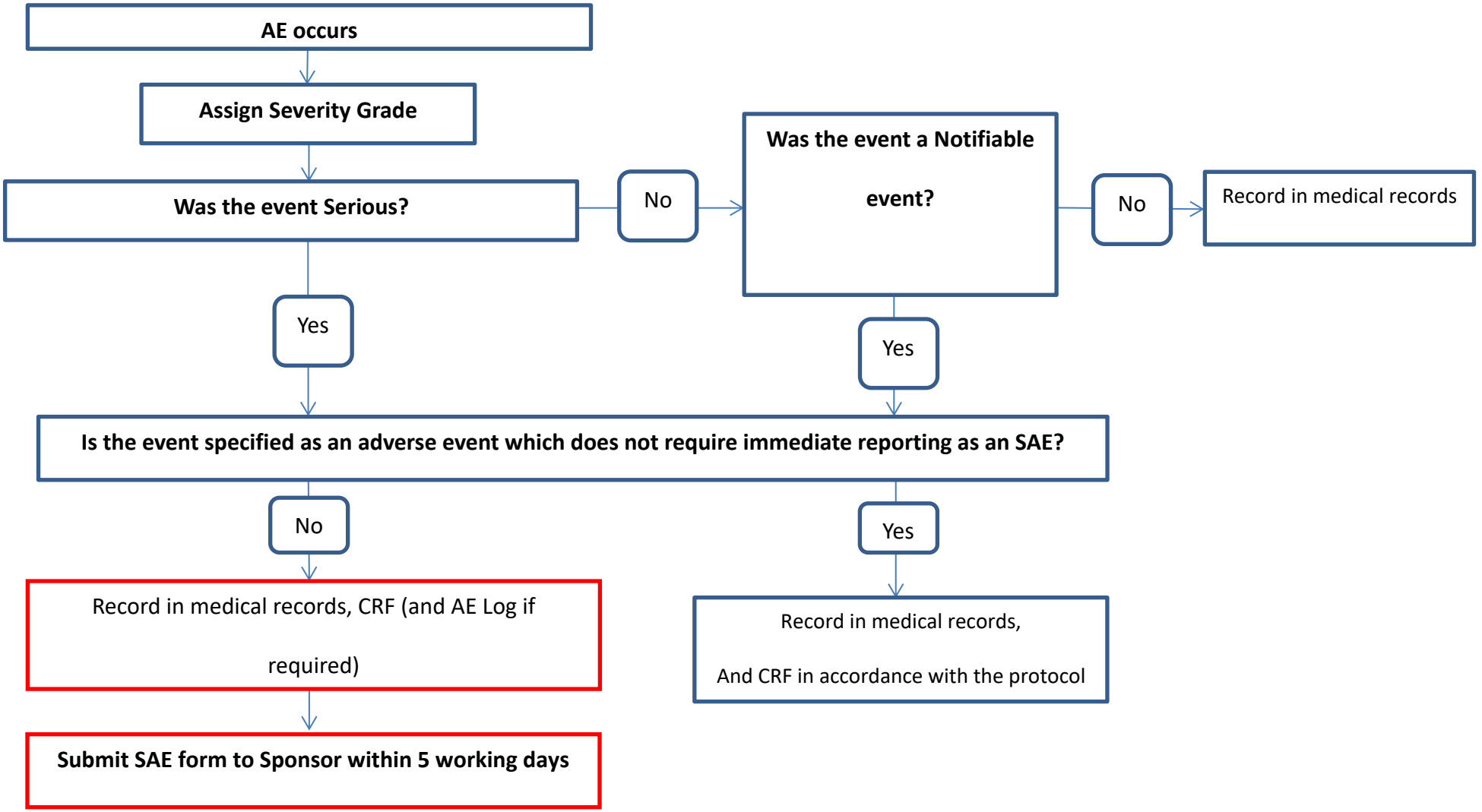
All SAEs (except those specified as not requiring reporting to the Sponsor) must be recorded on a serious adverse event (SAE) form. The CI/PI or designated individual will complete an SAE form and the form will be preferably emailed to the Sponsor within 5 working days of becoming aware of the event. The Chief or Principal Investigator will respond to any SAE queries raised by the sponsor as soon as possible.

Where the event is unexpected and thought to be related to the procedure this must be reported by the Investigator to the Health Research Authority within 15 days.

Completed forms for unexpected SAES must be sent within 5 working days of becoming aware of the event to the Sponsor

Email forms to: Research-incidents@ucl.ac.uk

Flow Chart for SAE Reporting:



Reporting Urgent Safety Measures:

If any urgent safety measures are taken the CI/ PI shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC and Sponsor of the measures taken and the circumstances giving rise to those measures.

Protocol Deviations and Notification of protocol violations:

A deviation is usually an unintended departure from the expected conduct of the study protocol/SOPs, which does not need to be reported to the sponsor. The CI will monitor protocol deviations.

A protocol violation is a breach which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the participants of the study; or
(b) the scientific value of the study.

The CI and sponsor will be notified immediately of any case where the above definition applies during the study conduct phase.

Trust Incidents and Near Misses:

An incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a. It is an accident or other incident, which results in injury or ill health.
b. It is contrary to specified or expected standard of patient care or service.
c. It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d. It puts the Trust in an adverse position with potential loss of reputation.
e. It puts Trust property or assets in an adverse position or at risk.

Incidents and near misses must be reported to the Trust through DATIX as soon as the individual becomes aware of them.

A reportable incident is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

a) It is an accident or other incident, which results in injury or ill health.
b) It is contrary to specified or expected standard of patient care or service.
c) It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
d) It puts the Trust in an adverse position with potential loss of reputation.
e) It puts Trust property or assets in an adverse position or at risk of loss or damage.

MONITORING AND AUDITING

The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting and ensure adequate data quality.

The Chief Investigator will inform the sponsor should he have concerns, which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

TRAINING

The Chief Investigator will review and provide assurances of the training and experience of all staff working on this study. Appropriate training records will be maintained in the study files

INDEMNITY ARRANGEMENTS

University College London holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

ARCHIVING

UCL and each participating site recognise that there is an obligation to archive study-related documents at the end of the study (as such end is defined within this protocol). The Chief Investigator confirms that he/she will archive the study master file at UCLH for the period stipulated in the protocol and in line with all relevant legal and statutory requirements. The Principal Investigator at each participating site agrees to archive his/her respective site's study documents for [insert duration] and in line with all relevant legal and statutory requirements.

PUBLICATION AND DISSEMINATION POLICY

Results shall be published by appropriate scientific journals if accepted. The data shall also be available in clinic for patients / participants possibly in the form of posters and/ or leaflets.

PARTICIPANT INVITATION LETTER

UCL HOSPITALS

University College London Hospitals NHS
NHS Foundation Trust

Short Title: Pubertal Induction (Student Study)
Version: 1.0
Date: 15/09/2016
IRAS: 199997
Title: Participant Invite Letter
R&D: 16/0332

Reproductive Medicine Unit
Women's Health
2nd Floor, North Wing
250 Euston Road
London
NW1 2PG

Email: elizabeth.burt@uclh.nhs.uk

Hospital No: XXXX
NHS No: XXXX
DOB: XXXX

Address XXX

Dear XXXXX

VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM.

I am writing to you as a person attending UCLH for management of your **(INSERT DIAGNOSIS)**. I would like to invite you to join a new research project investigating puberty induction.

The aim of the project is to see how your body changes as you go through puberty induction.

An information sheet about the project and your involvement is enclosed for you to read before your next appointment. During your next appointment you will be able to discuss the project with your doctor and decide as to whether or not you would like to join.

For further information or to have the relevant documents sent ahead of your clinical appointment contact Elizabeth Burt: elizabeth.burt@uclh.nhs.uk

Yours sincerely,

Professor Gerard Conway
Consultant Endocrinologist
Hon Professor of Clinical Medicine UCL

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INFORMATION SHEET FOR PARTICIPANTS AGED 16 AND OVER

Short Title: Puberty Induction (Student Study)

Version: 1.0

Date: 15/09/2016

IRAS: 199997

Title: Patient Information Sheet 16+

R&D: 16/0332

University College London Hospitals NHS
NHS Foundation Trust

Patient Information Leaflet for Participants 16 Years of Age and Over

VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM (STUDENT STUDY).

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it will involve for you. Please take time to read the following carefully and discuss it with others if you wish.

If you would like any further information or if anything is not clear, please ask us. Please take your time to decide whether or not you wish to take part.

What is the purpose of the study?

Some individuals, due to a range of different reasons, do not go through puberty naturally. They may not undergo breast development nor start their periods. If this is the case then they may need additional hormonal medications.

Prior to starting regular periods, the uterus (womb) has to grow and change shape. This occurs over many months and is a gradual process. Without oestrogen (female hormone) the womb will stay small and periods will not start. A way of looking at the shape and size of the womb is by ultrasound scan.

Oestrogen is also necessary for breast growth and development. Again, without hormones, the breasts will remain small. Examining breast growth and change in shape is a way to make sure there is enough oestrogen.

Some individuals, despite hormone (oestrogen) treatment, do not have good womb growth or breast development. Oestrogen is also necessary for bone health.

Why have I been invited?

You are being invited, as you may require medication to go through puberty

Do I have to take part?

Taking part in this research is entirely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and asked to sign a consent form. You are still free to withdraw from the study at any time and without giving a reason. A decision to withdraw at any time or not take part will not affect the standard of care you receive.

What will happen to me if I take part?

As part of your routine assessment:

- You will be reviewed in the clinic and the doctor will complete a medical assessment. At this time you will be given this information sheet and the consent form.
- We will ask you some questions about your medical history
- In addition, we will ask your permission to examine you. This will include measurements including your height and weight, blood pressure and your waist and hip measurement.
- We will ask to measure your body fat with a special machine called a Tanita.
- A member of the team, with your permission will examine your breast growth and your pubic and underarm hair growth.
- We will organise for you to have some blood tests taken.
- We may ask you to have an ultrasound scan which will be a standard gynaecology scan assessing the womb. This will be a transabdominal (tummy) scan. This will form part of the routine assessment. The womb will be measured, and the information will be recorded in the standard manner.
- To look at how healthy the bones are we will organise a scan of your bones called a DEXA scan.
- At a later appointment, as part of the routine assessment, we will also use an MRI scan to assess how well the womb has developed

As part of the research:

- In order to make sure that the measurements of your womb are accurate on ultrasound another person may repeat it.
- We are assessing other ways of measuring breast development. One way of doing this is to take special measurements using a 3D scan. A picture from you neck to under the ribs will be taken. No image will include your head and face. Images will be stored with only your hospital and study number.
- Blood tests may be sent to another hospital for analysis. Therefore additional blood tests will need to be taken (these can be taken at the same time as the other blood tests)
- Some of the blood tests may be stored so that we can use these at a later date.
- With your permission we will inform your GP of your treatment and that you are taking part in the study.

What medication will I need to take?

The doctor will talk to you about the medication that you will need to use to help you go through puberty. This medication would be the same if you took part in the research or not. The medication will be a patch (like plaster) that you stick on your abdomen/bottom/top of leg. You will need to cut the patch into either half or quarter to begin with and the doctor will show you how to do this. You can do your normal activity including swimming, exercising and washing with the patch on. If the patch were to come off then you can stick another patch on.

You will need to change the patch twice per week on a Thursday and Sunday. The doctor will be able to give you all the information with regards to this and answer any further questions you have.

How often will I need to come in?

As part of your standard care we would usually like to review how you are getting on every 3 months approximately. As part of the research you will be invited back every 2 months, at a time when it is convenient to you, either on a Monday or Friday to have a repeat general examination. This will mean that you will have an additional 2 visits to the hospital.

You may also require an ultrasound, blood tests and breast 3D scan – it will depend on your individual case.

Again, depending on your treatment plan, at future appointments we will ask your permission to look at your womb using an MRI scan and have a repeat bone scan.

After 4 visits (8 months) we will ask you to come back for a review every 3-6 months (as per routine care) depending on your particular treatment.

What will happen to the samples I give?

We will analyse some blood tests at UCLH in the standard manner. Additional blood samples may be stored and kept securely within the hospital. Some may be sent via a hospital courier to another hospital within the UK for specialist analysis, as these particular tests are not available at UCLH. During the study period stored samples will be labelled only with your hospital and study number for confidentiality purposes. We will keep some of the samples for up to 5 years in case other tests become available in the future.

What do I have to do?

If you are happy to participate in the study, then the consent form will need to be signed. You can participate even if you, are or have been, involved in other studies.

What are the disadvantages and risks of taking part?

As part of the routine care that you will receive you will need ultrasound scans to ensure that your womb is growing well. Scans are not usually uncomfortable. Another person repeating the scan to check the measurements will only take a few extra minutes.

Blood tests will also need to be taken and sometimes this can cause slight discomfort.

Having the images taken of the breast will not cause any discomfort and only takes a few minutes.

What are the side effects when taking part?

When we complete the ultrasound scans and blood tests, the doctors will inform you of the findings, as they would do routinely. If there is something unexpected identified, then the doctor will explain if any further testing is required and the process to do this.

What are the possible benefits of taking part?

There may be no immediate direct benefits for you as a participant, but the results from this research will increase our knowledge about puberty induction. You will also receive 2 additional assessments.

What happens when the research study stops?

We will publish our findings in journals for other scientists to read. As indicated above, at no time will you be identified.

Will my taking part in the study be kept confidential?

Your confidentiality will be a top priority throughout and after the study. The doctors involved in the research will collect the data.

The routine assessments (ultrasound scan, blood tests, DEXA bone scan, MRI) will be labelled with your identifiable information as these form part of standard care and medical records. This will be kept confidential as per hospital data protection.

Any data generated will be stored on the database maintained on secure hospital & university computers and only will be accessed by members of the research team.

What will happen if I don't want to carry on with this study?

When you are seen for the routine appointment if you do not wish to take part in the study, this will not in any way affect your planned treatment. Furthermore, you may choose to stop taking

part in the research at any time. Any information that has been already collected may still be used for the study purposes.

What if there is a problem?

If you are concerned about any aspect of this study, please speak to the researchers who will do their best to answer your questions. Please contact Elizabeth Burt via elizabeth.burt@uclh.nhs.uk. If you remain unhappy, you can make a formal complaint through the National Health Service (NHS) complaints procedure. Details can be obtained through the Details can be obtained through the University College London Hospitals (UCLH) Patient Advice and Liaison Service (PALS) on 0207 3447 3041, email: PALS@uclh.nhs.uk, address: PALS, Ground Floor Atrium, University College Hospital, 235 Euston Road, London, NW1 2BU.

University College London (UCL) holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise

What will happen to the results of the research study?

Some anonymous information may be shared with other doctors and researchers. Data sharing is important, to ensure research is open to peer scrutiny, to optimise the use of good quality research data and to support policy and other decision-making.

Sometimes information that we collect in this research maybe used in future research.

The results may be published in peer reviewed scientific journals. At any given point, you will not be identified in any report or publication. The results of the study will be utilised for completion of a MD thesis by a member of the research team.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, wellbeing and dignity. This study has been reviewed and given a favourable opinion by the _____ Research Ethics Committee.

Further Information and Contact Details:

If you would like to speak to anyone about this study please do not hesitate to contact, Dr Elizabeth Burt (Clinical Research Fellow Women's Health Division, University College Hospital) on elizabeth.burt@uclh.nhs.uk. Alternatively, you may discuss any questions or concerns arising from the study with Professor Conway (Consultant Endocrinologist & Chief Investigator) or with your doctor at your next Outpatient Clinic.

THANK YOU VERY MUCH FOR TAKING THE TIME TO READ THIS INFORMATION SHEET AND FOR CONSIDERING TAKING PART IN OUR RESEARCH STUDY.

INFORMATION SHEET FOR PARTICIPANTS UNDER THE AGE OF 16

Short title: Pubertal induction (Student Study)

Version: 1.0

Date: 15/09/2016

Title: Participant Information Sheet

IRAS: 199997

R&D: 16/X

University College London Hospitals NHS
NHS Foundation Trust

UCL

Patient Information Sheet for Participants Under the Age of 16

WHAT HAPPENS IF I NEED MEDICATION TO HELP MY BODY GO THROUGH PUBERTY?

We are asking whether you and your parents would be interested in taking part in a research project.

Before you decide if you would like to join, it is really important that you understand what the study is about, why the study is being done and what would be your involvement. So please read and think about this leaflet carefully. Also talk to your family, friends, doctors and nurses about it if you want.

If something is not clear or you have more questions you can ask your parents to contact us and we can discuss it with you and your parents.

Thank you for reading this.

Why are we doing this research?

Some people, due to lots of reasons, do not go through puberty naturally. They may not start breast development or their periods. If this is the case then they may need additional hormonal medications.

Prior to starting periods, the womb has to grow and change shape. This occurs over many months and is a gradual process. Without oestrogen (female hormone) the womb will stay small and periods will not start. A way of looking at the shape and size of the womb is by ultrasound scan.

Oestrogen is also necessary for breast growth and development and without hormones the breasts will remain small. Examining breast growth and change in shape is a way to make sure there is enough oestrogen.

Oestrogen is also necessary for strong bones.

In this study we will look at children and adults who have not gone through puberty naturally.

Why have I been invited?

You are being invited, as you have not started puberty or periods naturally.

Do I have to take part?

No you don't. It's your choice whether you take part and you can always change your mind.

What will happen to me if I take part?

We will invite you to the clinic in the hospital with at least one of your parents. You will meet the doctor and they will ask you and your parent some questions. You will have the opportunity to ask questions too. Then you and at last one of your parents will sign a form that they are happy for you to take part in the study.

The doctor will ask you about your health.

The doctor will measure your height, weight and blood pressure. They will ask to measure your waist and your hips. They will ask to have a look at your chest to see how much your breasts have developed and will look at the hair on your body. To look at your breast development, the doctor will ask to take an image of your chest, from your neck to belly button.

The doctor will ask you to have an ultrasound (jelly scan) of your tummy. To make sure the measurements are correct another doctor may also need to come into the room to do this again.

They will ask to take some blood tests and for you to have a scan of your bones. At a later appointment the doctor will ask to do an MRI scan of your tummy.

We will ask you to come back again, a few more times, to see how things are changing as a result of your medication.

What medication will I need to take?

The doctor will talk to you and your parents about the medication you that will need to use to help you go through puberty. The medication will be a patch (like plaster) that you stick on your tummy/bottom/top of leg.

Your parent will need to cut the patch into either half or quarter to begin with and the doctor will show them how to do this. You can do your normal activity including swimming, sport and washing with the patch on. If the patch were to come off then you can stick another on.

You will need to change the patch twice per week on a Thursday and Sunday.

The doctor will be able to give you and your parents all the information about this and answer any further questions you have.

Will any part of the study be uncomfortable or dangerous?

All parts of the study are safe. The blood tests can be a bit uncomfortable or give you a small bruise. The MRI scan can be noisy, but you can listen to music. Taking the image of the chest is quick and occurs in a private room. The bone scan and tummy scan are both safe and quick. The tummy scan may cause a very small amount of discomfort as we press on your tummy.

Will taking part in the study help me?

The study will not help you right now, but it will increase the knowledge for other people needing medication to go through puberty.

What do I do if I don't want to take part in the research anymore?

Just tell your parents and the people carrying out the research that you don't want to take part anymore. You don't need to give any reason. It is your choice.

What happens when the research study stops?

The results of the study will be written up so that other people can read about it, but they won't now that you were in the study. After the research stops you will carry on seeing your doctor and having treatment.

What if something goes wrong?

If there is a problem you should talk to your parents first and then any of the researchers.

Will my information be kept private? Will anyone else now that I am taking part?

All your information will be kept private. We will only inform those who have a need or right to know like your parents and GP.

What will happen to any samples that I give?

The blood samples that you give at your visits will be stored in a safe place. We will keep some of the samples for up to 5 years in case other tests become available in the future.

Who is organising and funding the research?

The study is being organised by University College London and will be taking place at University College London Hospital. There is no external funding.

Who have reviewed the study?

Before any research is allowed to go ahead it has to be checked by a group of people called the Research Ethics Committee. They make sure the research is fair. This study has been reviewed.

Further Information and Contact Details:

If you have any questions speak to your parents first. You can also speak to Professor Conway (Consultant Endocrinologist & Chief Investigator) or Dr Elizabeth Burt (Clinical Research Fellow) on elizabeth.burt@uclh.nhs.uk.

THANK YOU VERY MUCH FOR TAKING THE TIME TO READ THIS INFORMATION SHEET AND FOR CONSIDERING TAKING PART IN OUR RESEARCH STUDY.

INFORMATION SHEET FOR PARENT/GUARDIAN

Short Title: Puberty Induction (Student Study)

Version: 1.0

Date: 15/09/2016

IRAS: 199997

Title: Information for Parent/Guardian

R&D: 16/0332

University College London Hospitals NHS
NHS Foundation Trust

UCL

Information Leaflet for Parent/Guardian

VARIABILITY OF UTERINE AND BREAST DEVELOPMENT IN RESPONSE TO EXOGENOUS OESTROGEN DURING INDUCTION OF PUBERTY IN INDIVIDUALS WITH HYPOGONADISM (STUDENT STUDY).

We would like to invite your child to take part in a research study. Before you and your child decide you need to understand why the research is being done and what it will involve for your child. Please take time to read the following carefully and discuss it with others if you wish.

If you would like any further information or if anything is not clear, please ask us.

What is the purpose of the study?

Some individuals, due to a range of different reasons, do not go through puberty naturally. They may not undergo breast development nor start their periods. If this is the case then they may need additional hormonal medications.

Prior to starting regular periods, the uterus (womb) has to grow and change shape. This occurs over many months and is a gradual process. Without oestrogen (female hormone) the womb will stay small and periods will not start. A way of looking at the shape and size of the womb is by ultrasound scan.

Oestrogen is also necessary for breast growth and development. Again, without hormones, the breasts will remain small. Examining breast growth and change in shape is a way to make sure there is enough oestrogen.

Some individuals, despite hormone (oestrogen) treatment, do not have good womb growth or breast development. Oestrogen is also necessary for bone health.

Why has my child been chosen?

Your child is being invited to take part in the study as they require medication to go through puberty.

Does my child have to take part?

Taking part in this research is entirely voluntary. It is up to you and your child to decide whether or not to take part. You will be given this information sheet to keep and asked to sign a consent form. Your child will also receive an information sheet. You are free to withdraw your child from the study at any time and without giving a reason. A decision to withdraw at any time or not take part will not affect the standard of care your child will receive.

What will happen to my child if we agree to take part?

As part of the routine assessment your child:

- Will be reviewed in the clinic and the doctor will complete a medical assessment.
- Will be asked some questions about their medical history

- Will have measurements including height and weight, blood pressure and waist and hip measurement.
- Will have their body fat measured with a special machine called a Tanita.
- Will be examined to look at their breast growth and pubic and underarm hair growth.
- Will have some blood tests taken.
- Will have an ultrasound scan which will be a standard gynaecology scan assessing the womb. This will be a transabdominal (tummy) scan. The womb will be measured, and the information will be recorded in the standard manner.
- Will have a bone scan called a DEXA to look at how healthy the bones are.
- Will, at a later appointment, have an MRI to review womb growth and development.

As part of the research:

- In order to make sure that the measurements of the womb are accurate on ultrasound another person may repeat it.
- We are assessing other ways of measuring breast development. One way of doing this is to take special measurements using a 3D scan. A picture from the neck to under the ribs will be taken. No image will include the head and face. Images will be stored with only the hospital and study number.
- Blood tests may be sent to another hospital for analysis. Therefore additional blood tests will need to be taken (these can be taken at the same time as the other blood tests)
- Some of the blood tests may be stored so that we can use these at a later date.
- With permission we will inform your GP of the treatment and that your child is taking part in the study.

What medication will they need to take?

The doctor will talk to you and your child about the medication that they will need to use to help them go through puberty. This medication would be the same if they took part in the research not. The medication will be a patch (like plaster) that they stick on their abdomen/bottom/top of leg. The patch will need to be cut into either half or quarter to begin with and the doctor will show you how to do this. They can do their normal activity including swimming, exercising and washing with the patch on. If the patch were to come off then they can stick another patch on.

The patch needs to be changed twice per week on a Thursday and Sunday. The doctor will be able to provide all the information with regards to this and answer any further questions you have.

How often will I need to come in?

As part of the standard care we would usually like to review how your child is getting on approximately every 3 months. As part of the research, you and your child will be invited back every 2 months, at a time when it is convenient either on a Monday or Friday. This will mean that they will have an additional 2 visits to the hospital.

After 4 visits (8 months) we will review your child every 3-6 months (as per routine care) depending on their particular treatment.

What will happen to the samples they give?

We will analyse some blood tests at UCLH in the standard manner. Additional blood samples may be stored and kept securely within the hospital. Some may be sent via a hospital courier to another hospital within the UK for specialist analysis, as these particular tests are not available at UCLH. During the study period, stored samples will be labelled only with hospital and study number for confidentiality purposes. We will keep some of the samples for up to 5 years in case other tests become available in the future.

What do I have to do?

If you are happy for your child to participate in the study, then the consent form will need to be signed.

What are the disadvantages and risks of taking part?

Ultrasound scans form part of routine care to ensure that the womb is growing well. Scans are not usually uncomfortable. Another person repeating the scan to check the measurements will only take a few extra minutes.

Blood tests will also need to be taken and sometimes this can cause slight discomfort.

Having the images taken of the breast will not cause any discomfort and only takes a few minutes.

What are the side effects when taking part?

When we complete the ultrasound scans and blood tests, the doctors will inform you and your child of the findings, as they would do routinely. If something unexpected is identified, then the doctor will explain if any further testing is required and the process to do this.

What are the possible benefits of taking part?

There may be no immediate direct benefits for your child, as a participant, but the results from this research will increase our knowledge about puberty induction. They will also receive 2 additional assessments.

What happens when the research study stops?

We will publish our findings in journals for other scientists to read. As indicated above, at no time will your child be identified.

Will my child's taking part in the research project be kept confidential?

Confidentiality will be a top priority throughout and after the study. The doctors involved in the research will collect the data.

The routine assessments (ultrasound scan, blood tests, DEXA bone scan, MRI) will be labelled with identifiable information as these form part of standard care and medical records. This will be kept confidential as per hospital data protection.

Any research data generated will be stored on the database maintained on secure hospital & university computers and only will be accessed by members of the research team.

What will happen if my child or I don't want to carry on with the research?

If you or your child does not wish to take part in the study, you will be withdrawn from the study and this will not in any way affect their planned treatment. This can be done at any time. Any information that has been already collected may still be used for the study purposes.

What if there is a problem?

If you are concerned about any aspect of this study, please speak to the researchers who will do their best to answer your questions. Please contact Elizabeth Burt via elizabeth.burt@uclh.nhs.uk. If you remain unhappy, you can make a formal complaint through the National Health Service (NHS) complaints procedure. Details can be obtained through the Details can be obtained through the University College London Hospitals (UCLH) Patient Advice and Liaison Service (PALS) on 0207 3447 3041, email: PALS@uclh.nhs.uk, address: PALS, Ground Floor Atrium, University College Hospital, 235 Euston Road, London, NW1 2BU.

University College London (UCL) holds insurance against claims from participants for harm caused by their participation in this clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, if this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical study. University College London does not accept liability for any breach in the hospital's duty of care,

or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise

What will happen to the results of the research study?

Some anonymous information may be shared with other doctors and researchers. Data sharing is important, to ensure research is open to peer scrutiny, to optimise the use of good quality research data and to support policy and other decision-making.

Sometimes information that we collect in this research maybe used in future research.

The results may be published in peer reviewed scientific journals. At any given point, your child will not be identified in any report or publication. The results of the study will be utilised for completion of a MD thesis by a member of the research team.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, wellbeing and dignity. This study has been reviewed and given a favourable opinion by the local _____ Research Ethics Committee.

Further Information and Contact Details:

If you would like to speak to anyone about this study please do not hesitate to contact, Dr Elizabeth Burt (Clinical Research Fellow Women's Health Division, University College Hospital) on elizabeth.burt@uclh.nhs.uk. Alternatively, you may discuss any questions or concerns arising from the study with Professor Conway (Consultant Endocrinologist & Chief Investigator) or with your doctor at your next Outpatient Clinic.

THANK YOU VERY MUCH FOR TAKING THE TIME TO READ THIS INFORMATION SHEET.

CONSENT FORM FOR PARTICIPANTS AGED 16 AND OVER

University College London Hospitals NHS Foundation Trust

Short Title: Puberty Induction (Student Study)
Version: 1.0
Date: 15/09/2016
IRAS: 199997
Title: Consent Form
R&D: 16/0332

Reproductive Medicine Unit
Women's Health Division
North Wing, 250 Euston Road
London
NW1 2PG

PARTICIPANT CONSENT FORM FOR PARTICIPANTS 16 YEARS AND OVER

Variability of Uterine and Breast Development in Response to Exogenous Oestrogen During Induction of Puberty in Individuals with Hypogonadism (Student Study).

Name of Researcher _____

		Please initial box
1.	I confirm that I have read and understand the information sheet (Version 1 dated 15/5/2016) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3.	I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the UCLH NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my relevant records.	☐
4.	I agree to my GP being informed of my participation in the study.	☐
5.	I understand that blood samples may be stored for up to 5 years and may be used for future research	
6.	I understand that the information collected about me may be used to support other research in the future and may be shared anonymously with other researchers.	☐
7.	I agree to participate in this study.	☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

1
When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

ASSENT FORM FOR PARTICIPANTS UNDER THE AGE OF 16

Short Title: Puberty Induction (Student Study)
Version: 1.0
Date: 15/09/2016
IRAS: 199997
Title: Assent Form
R&D: 16/0332

ASSENT FORM FOR CHILDREN
(to be completed by the child and their parent/guardian)

WHAT HAPPENS IF I NEED MEDICATION TO HELP MY BODY GO THROUGH PUBERTY?
(Student Study)

Child (or if unable, parent on their behalf) /young person to circle all they agree with please:

Have you read (or had read to you) about this project?	Yes/No
Has somebody else explained this project to you?	Yes/No
Do you understand what this project is about?	Yes/No
Have you asked all the questions you want?	Yes/No
Have you had your questions answered in a way you understand?	Yes/No
Do you understand it's OK to stop taking part at any time?	Yes/No
Are you happy to take part?	Yes/No

If any answers are 'no' or you **don't** want to take part, **don't** sign your name!

If you **do** want to take part, please write your name and today's date.

Your name _____

Date _____

Your parent or guardian must write their name here too if they are happy for you to do the project

Print Name _____ Sign _____

Date _____

The doctor who explained this project to you needs to sign too:

Print Name _____
Sign _____
Date _____
Thank you for your help.

CONSENT FORM FOR PARENT/GUARDIAN

University College London Hospitals NHS
NHS Foundation Trust

Short Title: Pubertal Induction (Student Study)
Version: 1.0
Date: 15/09/2016
IRAS: 199997
Title: Consent Form
R&D: 16/0332

Reproductive Medicine Unit
Women's Health Division
North Wing, 250 Euston Road
London
NW1 2PG

Parent/Guardian Consent Form

Variability of Uterine and Breast Development in Response to Exogenous Oestrogen During Induction of Puberty in Individuals with Hypogonadism (Student Study).

Name of Researcher _____

	Please initial box
I confirm that I have read and understand the information sheet (Version 1 dated 15/5/2016) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I agree for my child to take part in the study.	☐
I understand my child's participation is voluntary and that they are free to withdraw at any time, without giving any reason and without their medical care or legal rights being affected.	☐
I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from regulatory authorities, from UCLH NHS Trust or UCL, where it is relevant to their taking part in this research. I give permission for these individuals to have access to their relevant records.	☐
I understand that blood samples may be stored for up to 5 years and may be used in future research	☐
I agree to their GP being informed of my child's participation in the study	☐
I understand that the information collected may be used to support other research in the future and may be shared anonymously with other researchers.	☐

_____	_____	_____
Name of Parent/Guardian	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

APPENDIX 4. HEALTH RESEARCH AUTHORITY APPROVAL LETTER

NHS
Health Research Authority

Professor Gerard Conway
UCL
Gower Street
London
WC1E 6BT

Email: hra.approval@nhs.net

28 March 2017

Dear Professor Conway

Letter of HRA Approval

Study title:	**Variability of uterine and breast development in response to exogenous oestrogen during induction of puberty in individuals with hypogonadism.**
IRAS project ID:	**199997**
REC reference:	**17/SW/0047**
Sponsor	**University College London (UCL)**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

APPENDIX 5. FERTILITY AND PREGNANCY TELEPHONE INTERVIEW

Note: This is intended for women with POI or TS who are known to have had fertility treatment or pregnancy. Allocate a Study ID and record name and identifiers on a local secure listing.

How old are you?

How old were you when TS/POI was diagnosed?

Have you ever had treatment for the following (details)?

Hypertension	Diabetes
Hypothyroidism	Depression
Osteoporosis	Heart disease
Hearing loss	Other

In the family, does anyone suffer from the following?

High blood pressure
Diabetes
Thyroid problems

TS only: Do you know your karyotype (details)?

Do you have a bicuspid valve?

Did you ever receive growth hormone (details)?

Are you taking HRT?

What type of HRT are you taking?

At what age did you start?

What was the first type of HRT you ever took?

How old were you when you had your first period (natural or induced)?

Before fertility treatment, had you ever had an ultrasound scan of the uterus?

If so where and at what age and where?

How tall are you? How much do you weigh?

Before the first fertility treatment or pregnancy….

Had you recently attended a hospital clinic (details)?
What type of oestrogen were you taking just before treatment or pregnancy?
Had adequate an opportunity discussed fertility options?

TS Only: Had you discussed pregnancy risks?

Had you had a recent echocardiogram?

Had you seen a cardiologist?

Cardiology details:

Fertility treatment Data Collection for Telephone Interview.

Note: one line for each attempt – insert NK if not known

Centre ID Subject ID

Attempt Number	Age at Attempt	ART Clinic / Country	NHS or Private?	Donor Details e.g. Anon, Relative, Friend, Bank Egg share	Donor Age	Total No. Embryos Created	Number Embryos Transferred	Stage of Embryos Transferred	Fresh or Frozen	Cycle Outcome e.g. Misc, Ectopic, Live Birth, Not preg

Free text for notes and clarifications:

Pregnancy Data Collection for Telephone Interview.

Note: one line for each pregnancy including miscarriages and ectopic – insert NK if not known

Centre ID Subject ID

Preg No	Age at Preg	Mode of conception e.g. Spont, OD, IVF	Fetus no.	Preg test Fetal heart? Biochem only?	Pregnancy Outcome e.g. Misc (add week of misc), Live birth, Ectopic	Pregnancy complication?	HTN y/n	GDM y/n	PET y/n	Mode of delivery e.g. Vaginal, If CS – planned or emergency	Obstetric Unit & Delivery Complications	Gest Age	Baby Sex & Wt (kg)	Birth defects Baby health	Breast Feeding Details and successful no of weeks

Free text for notes and clarifications: