ENVIRONMENTAL EFFECTS ON OVARIAN RESERVE AMONG MIGRANT BANGLADESHI WOMEN IN THE UK

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THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

2011

DECLARATION

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

DEDICATION

To my beloved husband

ACKNOWLEDGEMENTS

The study presented in this thesis was made possible by the help and guidance I received from a number of people, as well as numerous collaborators.

At the very outset, I would like to express my sincere gratitude to my principal supervisor, Prof. Gillian R Bentley for giving me the opportunity to carry out this promising research. Her valuable teaching, continuous guidance, supervision, tolerance, constructive criticism and all out support made this study possible. I have learnt a lot from her. Thanks a million for everything!

My heartfelt thanks go to Prof. Lynnette Leidy Seivert for believing in me that I could make it and for her support. It was a great privilege to work under her supervision in Bangladesh.

Sincere thanks to my secondary supervisor, Dr. Shanthi Muttukrishna, who supervised and helped in my laboratory work and also for her constructive criticisms and suggestions.

I am grateful to Prof. Osul Chowdhury for providing facilities and support to conduct field work in Bangladesh.

I am indebted to the women (study subjects) who generously dedicated their time and contributed blood as healthy volunteers. Without the contributions of these women this research would not have been possible.

My gratitude to Nazim Bhai and Rina Bhabee for helping me in recruiting women in Sylhet.

My outmost appreciation to the members of the Community Centres in Camden and East London for their help in the recruiting process, particularly to Syeda Rekha Faruk at Lime House Project, Counsellor Rohima Rahman at Katherine Community Centre, Sheouli Khatun at Kollun Women's Centre, and Dr. Monowara Rahman, Ashrafunnessa Haque, Jashon Hussain, Khairun and Nahid Pervin at Surma Community Centre.

I am grateful to Kesson Magid for being always keen to share his knowledge, time and who has been a fantastic friend over the past four years and long into the years to come.

I would like to thank Diana Goforth and Chris Hagisavva for making me welcome and the congenial atmosphere in the department.

Special thanks to my family and my son for their sacrifices. Finally, and most importantly, I am proud of my husband Dr. Shahidul Mannan for his constant encouragement and sacrifice throughout the study period.

I am indebted to the generosity of the National Science Foundation for providing with me the scholarship for this study.

ABSTRACT

Reproductive ecologists have proposed that environmental conditions experienced during development influence adult reproductive hormones. An earlier study on Bangladeshi women aged 18-35 showed that women who migrated to the UK during childhood (<16 years) have significantly higher salivary progesterone levels compared to women who grew up in Bangladesh. But no such study has been reported in the context of later reproductive hormone levels and ovarian reserve. In the research here, hormone profiles that predict ovarian reserve (inhibin B, AMH and FSH) were compared between: 1) migrant Bangladeshis who moved to the UK as adults, or 2) migrant Bangladeshis who moved as children, 3) sedentee Bangladeshis living in Bangladesh, and 4) white European women. Data on socio-economic, demographic and reproductive histories were also collected. The following hypotheses were examined: 1) There is inter-population variation in ovarian reserve depending on environmental conditions during development; 2) Moving to a better environment during adult life does not affect age-specific ovarian reserve; and 3) The childhood environment has an impact on age-related ovarian reserve in later life. The findings support these hypotheses. Results suggest that changes in the developmental environment during childhood, when the tempo of growth and maturation are determined, influence reproductive hormone levels and ovarian reserve. Conversely, environmental change during adult life, when maturation is completed, does not alter later life reproductive hormone levels or ovarian reserve. The childhood environment therefore appears to have a significant effect on ovarian reserve reinforcing earlier findings that developmental plasticity extends beyond the uterine period in humans. Consequently, the higher age-specific ovarian reserve of child migrants who grew up in the UK results in an extended reproductive life span compared to women who grew up in Bangladesh. This may eventually put child migrants at an increased risk of developing age-related diseases such as breast cancer.

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PREFACE

Despite being a universal female phenomenon, reproductive ageing exhibits variation across different ecological settings. Therefore, understanding variation in reproductive ageing, ovarian reserve (number of viable follicles in the ovary at any given time) and hormone levels between populations in the menopausal transition is a key issue for the reproductive biologist. Several factors affect ovarian reserve and the reproductive ageing process including genetics, environmental factors, socio-economic conditions, psychological and stressful living conditions and reproductive life (such as fertility, sub-fertility, number of pregnancies, sexually transmitted infections, pelvic inflammatory disease).

Various studies suggest a marked variation in reproductive steroid levels in women of reproductive age living in highly stressful environments (e.g., with poor nutritional intake, high energetic output, and/or a high disease load). Poor growth and maturation in early life result in persistent lower baseline levels of salivary progesterone in adulthood (Ellison, 1966). Nunez de la Mora et al. (2007) have demonstrated that Bangladeshi sedentees and young Bangladeshi women (aged 18-39) who migrated to London as adults have low salivary progesterone profiles compared to higher levels among child migrant, second generation Bangladeshi women born in the UK and similarly aged neighbouring European women, but no significant differences were detected among similarly aged adult migrant and sedentee Bangladeshi women (Nunez et al. 2008). However, it is not well understood whether this discrepancy persists in later life and if it can affect age-related ovarian reserve.

It is now well established that three specific reproductive hormones (follicle stimulating hormone (FSH), inhibin B and anti-müllerian hormone (AMH) can predict ovarian reserve. In this thesis, I will compare hormonal variation reflecting ovarian reserve among sedentee Bangladeshis, migrant Bangladeshis who moved at different stages of the life course, and a comparative group of women of European descent in London matched for socioeconomic status.

The overarching question that connects the issues addressed in this thesis is: what role do *ecological factors* play in giving rise to differences in ovarian reserve and changes in ovarian and pituitary hormonal levels between populations? The issues to be addressed here relate to patterns of hormonal changes seen in women with increasing age, with particular emphasis on the migrant Bangladeshi (both adult and child) population in London and the effects of ecological factors on changes in hormone levels among sedentee and migrant Bangladeshis.

Therefore the hypotheses and predictions of the study are as follows:

Hypothesis 1: As early life developmental conditions impact on later life reproductive hormonal levels, there is inter-population variation in reproductive hormone levels as well as ovarian reserve. Therefore, women who grow up in an adverse environment will have lower age-related ovarian reserve compared to women who grow up in a better environment.

Prediction 1: Bangladeshi women who grew up in Bangladesh will have a lower ovarian reserve compared to women of European descent.

Hypothesis 2: Growing up in an adverse environment and migration to a better environment during adult life does not affect ovarian reserve.

Prediction 2: Women who grew up in Bangladesh and migrated to the UK as adults will have ovarian reserve that is comparable to sedentees.

Hypothesis 3: The childhood environment has an impact on age related ovarian reserve in later life. Therefore, migration to a better environment during childhood will result in a higher ovarian reserve compared to women still in the community of origin and adult migrants.

Prediction 3: Migrant Bangladeshi women who migrated in the UK during childhood will have a higher ovarian reserve compared to sedentees and adult migrant Bangladeshi women who spent their childhood in Bangladesh. In other words, Bangladeshi migrants who moved to the UK as children have later decline in ovarian reserve compared to women who grew up in Bangladesh.

To set the stages for answering these questions, Chapter One therefore gives background into reproductive physiology and ovarian reserve to explain the importance of using biomarkers to assess Ovarian Reserve. Chapter Two gives the background into life history theory to place in context an evolutionary framework in which to understand why the childhood environment might be important for influencing/altering reproductive function in individuals, and a brief outline of possible mechanisms behind the altered reproductive function. Chapter Three provides an overview of the history and background of the Bangladeshi community in the UK and reports on socio-demographic trends among migrants

in comparison to the reference groups. Chapter Four describes the methodology that provides information relating to data collection procedure, laboratory techniques for hormonal assays and information on the statistical analyses used. Chapter Five focuses on the results of the study and provides information on the representativeness of the sample population in relation to larger project, the distribution of socio-economic and bio-characteristics of the sample population, the findings of the hormonal assays and results from testing the hypotheses. Chapter Six comprises a discussion of the hypothesis in the light of the study findings, explains the findings in the context of present knowledge and existing theories and hypotheses, a conclusion, and a section on the importance of the study and future research.

This is the first study to include hormonal assays to predict ovarian reserve during the menopausal transition, and post menopausal period within the context of an international migration study comparing migrants, the local host community, and the community of migrant origin. The data generated by using these hormonal biomarkers will help in future studies on reproductive ageing at the population level. Although biomarkers have been used before in measuring ovarian reserve, they are mostly studied in the context of assisted reproductive technologies (ART). This research gives a new perspective on the use of biomarkers for measuring reproductive ageing at the population level and gives new information concerning variability in reproductive ageing as well as the causes of this variability. It extends our knowledge on the importance of the childhood developmental period for determining reproductive function in later life. The findings give comparative information on reproductive ageing among the Bangladeshi migrant population compared to sedentee Bangladeshis and their European neighbours. Thereby it increases

our knowledge of reproductive health issues for the ethnic minority population in the UK. Finally this study might give a better understanding from the clinical perspective to variation in age-related health problems like osteoporosis, coronary heart disease and breast cancer of ethnic minority women.

Candidate's contribution:

This study is a part of larger collaborative project between University College London (UK), Durham University (UK), University of Massachusetts-Amherst (USA) and Sylhet Osmani Medical College (Bangladesh). Under the supervision of Prof. Gillian R Bentley, Professor of Anthropology, Durham University, UK, the candidate was fully involved in all stage of the present study. KB participated in designing the data collection strategy, networking with the Bangladeshi community both in London and Sylhet and coordinating the organisation and logistics of fieldwork. I helped to design and pilot the questionnaires and ran health sessions and workshops in several Bangladeshi community centres; carried out all the recruitment, interviews and collection of blood samples of the women for this study; performed hormonal assays in the laboratory under the supervision of Dr. Shanthi Muttukrishna in the Department of Obstetrics and Gynaecology, UCL, UK and was responsible for the data coding, entering, cleaning and analyses. Finally, I wrote up the contents of this report e.g. background, statistical analysis and interpretation, and discussion of the findings.

CHAPTER 1

REPRODUCTIVE PHYSIOLOGY, OVARIAN RESERVE AND REPRODUCTIVE AGEING

1.1 Female reproductive life: an overview

A long period of dependence on parents in early life, slow physical growth, delayed commencement of sexual maturity and having an unusually long life span are relatively unique characteristics of human development. As a result, reproduction starts late due to the extended period of childhood dependency compared to other social mammals and primates (Bogin and Smith, 1996), continues over several decades, and terminates several years before death (Wood, 1994). Female reproductive life is characterised by regularly recurring cycles commonly called "menstrual cycles" (as menstrual bleeding is the obvious feature) during which women can conceive and establish a pregnancy. However, physiologists use the term "ovarian cycle" because the principal actress in many respects is the ovary. In a normal cycle, there is brief period at mid-cycle when conception may result if insemination occurs.

A normal ovarian cycle includes development of an ovarian follicle, release of the ovum from the follicle, and formation and regression of the corpus luteum from the remaining follicle cells.

The cycle has three phases, starting with the menstrual phase when menstrual bleeding begins, followed by a follicular phase when the final stage of oocyte development is completed with release of the ovum and, finally, the luteal phase when the endometrium of the uterus is prepared to accommodate a potentially fertilised ovum. Although the cycle length of a normal menstrual cycle is generally considered as 28 days, studies on menstrual cycles of women of reproductive age suggest an average length of 29 ± 7.5 days (Chizze et al., 1968; Odujlhrin and Ekunwe, 1991). However, the lengths of women's menstrual cycles typically vary with some having shorter cycles and some longer ones. A variation of less than ten days between shorter and longer cycles in a woman is considered to be a regular menstrual cycle. It is usual for a woman to experience cycle length variation of less than 4 days. A variable length cycle between a woman's shortest and longest cycle from 8-20 days is considered as moderately irregular, while variation of 21 days or more is considered as very irregular menstruation (Kippley and Kippley, 1996).

In a normal menstrual cycle, ovulation occurs at around day 14 of the cycle when the mature follicles ruptures and releases the ovum that is ready to be fertilised if insemination occurs. In some instances, ovulation fails to occur during the menstrual cycle in

women and is thus called an anovulatory cycle. Such cycles are quite common following menarche, during the early months of resumption of post-lactational or post-partum cycling, and also before the onset of menopause. It also occurs even among otherwise normal women. One study showed a 22% anovulation rate among healthy women aged between 20 and 31 years (Vuorento et al., 1989). Another more recent study involving 65 healthy adolescent girls aged 14-19 suggested that about one-third of them had anovulatory cycles (Vuorento and Huhtaniemi, 1992).

The menstrual interval during anovulatory cycles is variable, but is usually less than 28 days from the last menstrual period. The bleeding is also variable and ranges from scanty to relatively profuse (Ganong, 2005). The menstrual bleeding in an ovulatory cycle occurs as a result of a decline in progesterone levels due to degeneration of the corpus luteum. This decline in progesterone means the endometrial lining in the uterus is no longer maintained and therefore sloughs off and leads to menstrual bleeding. Therefore, bleeding in an ovulatory cycle is from progesterone withdrawal and its consequences. In contrast, in an anovulatory cycle, there is no corpus luteum formation due to a lack of progesterone. Bleeding occurs in an anovulatory cycle because oestrogen also contributes to endrometrial growth and the decline

in oestrogen levels at the end of even an anovulatory cycle also leads to the breakdown of the endometrial lining. Menstrual bleeding in such a cycle is, therefore, from oestrogen withdrawal (Ganong, 2005).

Onset of menstruation (menarche) marks the commencement of the reproductive phase of women, which ends with menopause. Studies on the reproductive function of healthy women during their reproductive phase have found a progressive shortening of the follicular phase length, which is longest during a woman's early twenties and shortest during her forties (Lenton et al., 1984). The trajectory of female reproductive function reveals that fecundity gradually increases after menarche and continues to increase till the mid-twenties, followed by a gradual decline until ovarian reserve is completely depleted (Ellison 1989). Women, however, remain fertile generally until the end of their third decade of life.

Although this three phase picture of female reproductive function across the decades is familiar in every population, there is considerable variation in fertility among naturally fertile populations Studies on Boston women (Lipson and Ellison 1992), Lese women of Zaire (Bailey et al., 1989; Ellison 1989), Tamang Nepali women (Panter-Brick, 1993) have found significant variation in hormonal (salivary progesterone) levels across age

groups (Ellison, 1994). Female reproductive function is hypothesised to occur along a broad continuum of variation both within and between individual populations. It is suggested that these patterns can result in potentially longer waiting times to conception for individual women with lower levels of reproductive steroids (Ellison, 1991).

The role of reproductive and metabolic hormones in affecting life history processes is crucial. They determine reproductive efficiency and control the growth rate and timing of development such as puberty by modifying metabolism and setting internal regulatory factors. During puberty, reproductive capability is established through processes initiated by neuroendocrine mechanisms (Worthman, 1999). Therefore, measurement of hormones provides a window on key determinants to ongoing function, adaptation, and differential well being. Furthermore, comparative studies of individual- and population-level endocrine variation shed light on ecological determinants of such variation and provides a much broader basis with which to characterise normal human biology.

1.2 Endocrine trajectory of reproductive development

The normal course of endocrine function for reproductive development is regulated through the hypothalamic-pituitary-

gonadal axis (HPG axis) (Ganong, 2005; Worthman, 1999) (Figure 1.1), which is centrally controlled by the hypothalamus through its secretion of gonadotropin-releasing hormone (GnRH). GnRH stimulates the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinising hormone (LH). Gonadal activity, including oestradiol (E2) production in females, is controlled by the amount and pattern of FSH and LH, which are regulated through a feedback mechanism exerted by blood levels of these steroids on the anterior pituitary gland and hypothalamus. The HPG axis plays two important roles: regulation and maintenance of adult reproductive function, and control of reproductive development and senescence. The timing of reproductive events is a key element in life history strategy and reproductive success (Ganong, 2005; Worthman, 1999).

Neuroendocrine-endocrine interactions play key roles in human life history, including the prolonged period of reproductive immaturity during early life, the relatively later onset of puberty. and an early reproductive senescence in women relative to lifespan. Both inter- and intra–population variation in the timing of puberty exists that has both genetic and environmental bases including nutrition, infections and gestational factors (Worthman, 1999). The characteristic features of HPG activity by age are as follows: early gonadal quiescence, activation at puberty that

supports adult reproductive function through hypothalamic control, and reproductive senescence due to gonadal ageing.

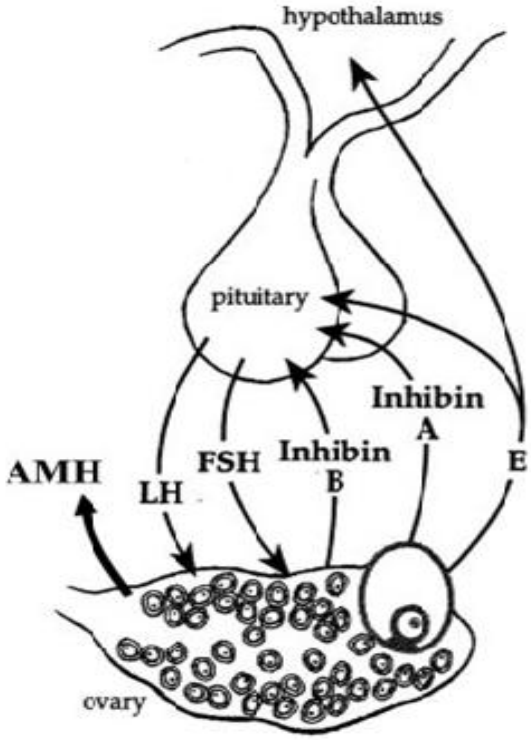
1.3 Biology of the normal ovarian cycle

For regular ovulatory cycles, changes of concentration of several hormones occur in a women's circulation, which play a critical role in the control of the ovarian cycle. The hormonal control of the cycle is regulated through a closed-loop, endocrine feedback system (the hypothalamic-pituitary gonadal axis) in which pituitary gonadotropin secretion is under negative feedback control by ovarian steroids and inhibin B. The steroids exert negative feedback effects on both FSH and LH (except at mid-cycle), whereas inhibin B specifically inhibits FSH production. The endocrinology of the menopause can be summarized as a series of phenomena that results in replacement of the cyclical secretory pattern of gonadotropins and gonadal steroids and peptides by a pattern of elevated gonadotropins and low levels of E2, progesterone and inhibin B.

The ovarian cycle is controlled by a complex interplay of different hormones, hormone-binding proteins and receptors. Hormones that control the ovarian cycle fall mainly into two classes: the gonadotropins and ovarian steroids (Figure 1.2). The pituitary gonadotropins are LH and FSH, and the ovarian steroids

are E2 and progesterone. The other factors are peptides that belong to the transforming growth factors (TGF) β super-family. Inhibin B and anti-müllerian hormone (AMH) are two protein hormones of the TGF-β super-family, which are released by the follicles and play a major role during the ovarian cycle. The concentrations of both gonadotropins and ovarian hormones in the blood undergo characteristic changes over the course of the cycle (Ganong 2005; Wood, 1994).

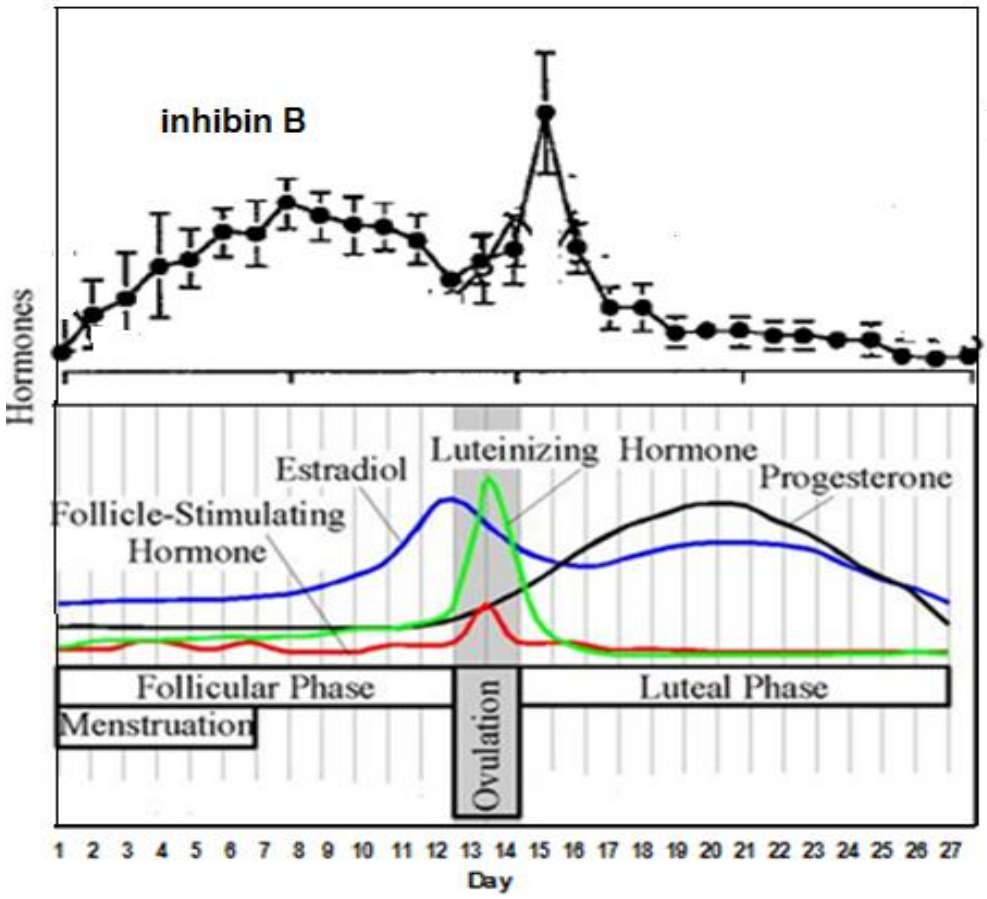
Figure 1.1 Diagram of hypothalamo-pituitary-gonadal axis illustrating sites of secretion of reproductive hormones, and the negative (-) and positive (+) feedback loops. (Adapted from Soules et al., 1998)



FSH and LH are two glycoprotein hormones that are released from the anterior pituitary gland. In females, FSH

stimulates ovarian follicular growth and LH stimulates ovulation and luteinisation (the process of transformation of the postovulatory ovarian follicle into a *corpus luteum* through vascularisation, follicular cell hypertrophy, and lipid accumulation) of the ovarian follicle. Both FSH and LH persist in the blood at low concentrations at the commencement of the ovarian cycle, but a slight elevation in FSH levels occurs in the late luteal phase and early follicular phase. Levels of LH and FSH remain low throughout most of the follicular phase until a day before ovulation when they rise suddenly (called the pre-ovulatory surge) but transiently. Both hormones come back to previous levels by the time of ovulation, and remain at this low level throughout the luteal phase. This pre-ovulatory surge is responsible for the release of the mature ovum into the abdominal cavity and thence to the fallopian tubes for potential fertilisation. FSH levels rise with increasing age and are negatively correlated with inhibin B (Muttukrishna et al., 2000). This secondary rise in pituitary FSH production is due to a decreased production of inhibin B by the immature follicles of the ovary, which results in withdrawal of suppressive action on the negative feedback loop.

Figure 1.2 Cyclical patterns of changes in the hormonal levels in a normal ovarian cycle (adapted and modified from Muttukrishna et al. 1994 and Wikipedia- http://en.wikipedia.org/wiki/Menstrual_cycle**)**



The ovarian steroids are E2 and progesterone. E2 is secreted primarily by the granulosa cells of the growing ovarian follicle, the corpus luteum, and the placenta during pregnancy. On the other hand, progesterone is primarily secreted by the corpus luteum, the placenta in pregnant women and by the follicle in small amounts. E2 is primarily responsible for ovarian follicular growth, while the principal target organ of progesterone is the uterus. The blood levels of both steroids undergo a characteristic pattern of change throughout the cycle. At the beginning of the follicular phase both steroids levels are very low. Blood levels of

E2 rise transiently just before ovulation and come down to baseline after ovulation, and a further rise occurs in the middle of the luteal phase. In contrast, blood progesterone levels remain at a very low level throughout follicular phase, but gradually start increasing immediately after ovulation and reach peak levels in the middle of the luteal phase. If conception does not occur, progesterone levels decline to undetectable levels at the end of the luteal phase In anovulatory cycles, low E2 levels can lead to either a low or no LH surge thus preventing ovulation. Furthermore, lack of ovulation leads to low progesterone levels due to failure to develop a corpus luteum and the absence of a luteal phase Therefore, in an anovulatory cycle, there are low E2, LH and progesterone levels.

A two-way signalling system between the oocyte and granulosa cells, and granulosa cells and theca cells is essential for the progression of follicular development through the different stages. The major role of this communication is played by the TGF-β super-family (Eppig, 2001), a group of structurally conserved but functionally diverse proteins. The members of this super-family are divided into several sub-families include TGF-β subfamily, bone morphogenic factor (BMP) sub-family, growth and differentiation factors (GDF) sub-family, inhibin/activin subfamily and other members like AMH.

The members of the TGF-β super-family are expressed by the oocytes and granulosa cells of the developing follicles in a sequential manner to the development of ovarian follicle (Erickson and Samasaki, 2003). They play key roles in different phases of follicular development, including recruitment of follicles, proliferation of granulosa and theca cells, steroid production, receptor expression, maturation of the oocyte, ovulation, luteinisation and *corpus luteum* formation. The later stages of follicular development including follicle selection are also dependent on the timing of endocrine secretion, receptor and locally produced factors of the TGF-β super-family.

The concept of an inhibitory factor that regulates FSH secretion by the anterior pituitary through feedback mechanisms was first postulated by McCullagh in 1932 as a part of research on infertile males. Several studies have suggested the presence of inhibitory regulation of FSH (Wallach et al., 1970; Franchimont et al., 1972; Wise et al., 1973). Later in 1975, while studying the changes in LH, FSH, E2 and progesterone levels of the normal women at two extremities of the reproductive life (menarche and menopause), Sherman and Korenman (1975) have found that premenopausal women had increased FSH levels with constant LH levels suggesting a different factor (inhibin B) regulating FSH secretion.

The inhibins are glycoprotein hormones produced by the ovary that exist as hetero-dimers (molecules made up of two simpler non-identical molecules) consisting of inhibin A and inhibin B. Inhibin B is mainly produced by small antral follicles in the ovary in response to gonadotropin stimulation (Roberts, 1993). Serum inhibin B levels are at their highest in the early and late follicular phases, fall in the periovulatory phase, and are at their lowest in the mid- and end-luteal phases (Groome et al., 1994). The postovulatory inhibin B peak occurs 1-2 days after the LH peak. This inhibin B peak is probably derived from follicular fluid released during ovulation. With respect to timing during the luteal-follicular transition, Groome et al. (1996) described inhibin B levels as being unchanged on the day of the FSH peak, but rising thereafter to peak 4 days later. Inhibin B levels reduce with increasing age and indicate the reduction of ovarian reserve (Muttukrishna et al., 2000).

AMH is mainly secreted by granulosa cells of the growing follicles (Baarends et al., 1995), and is not controlled by the gonadotropins (Van Rooij et al. 2002). It begins production immediately after differentiation of the primordial follicles from pre-granulosa cells to granulosa cells. Secretion of AMH by the follicle rises with the advancement of follicular development, reaches its peak in granulosa cells of preantral and small antral

follicles, and falls thereafter in the subsequent stages of follicle development. Therefore, this hormone appears first when follicles start developing into primary follicles, reaches a peak when follicles becomes preantral and small antral follicles (≤4 mm), and disappears when the follicular size increases to larger than 8 mm (Weenen et al. 2004). In a mouse model, Durlinger et al. (2002) showed that fewer follicles are recruited in cultured neonatal ovaries in the presence of AMH than in absence of AMH. These findings suggest that AMH inhibits further recruitment of the follicle. However, several studies on inter-cycle variability found that AMH does not vary significantly between the cycles (Streuli et al., 2008), which suggests a non cyclic pattern in serum AMH level throughout the menstrual cycle. As the follicles are recruited continuously from the resting pool to the growing pool, serum AMH levels in the follicular phase do not vary. Reports indicate that AMH levels decline with increasing age (de Vet et al., 2002; Hansen et al., 2003; Bancsi et al., 2004; Elter et al., 2005; Streuli et al., 2008). Therefore, serum AMH serves as a good endocrine marker for ovarian reserve.

1.4 Ovarian reserve and reproductive ageing

Human female reproductive life starts late and continues for several decades, but terminates several years to decades before death (Wood, 1994). This results in a post reproductive period

termed "menopause", a characteristic of human female reproductive life which extends across nearly one third of their lives in affluent human populations (Treloar, 1981). Physiologically, change occurs in the reproductive system in relation to age (reproductive ageing) and is reflected by a decline in the size of the primordial follicle pool (ovarian reserve). The ultimate effect of reproductive ageing on the female reproductive system is menopause, manifested by a complete cessation of menstruation as the ovaries stop producing steroid hormones. The whole process occurs gradually, taking several years (about an average duration of 4 years) in most women. The process starts before the establishment of menopause and is known as the menopausal transition, or premenopause. It is generally agreed that menopause is defined by permanent cessation of menstruation resulting from the loss of ovarian follicular activity and is recognized to have occurred after 12 consecutive months of amenorrhea for which there is no other obvious pathological or physiological cause (WHO, 1981; WHO, 1996; Leidy, 1999). The stages of the menopausal process are: premenopause (women who have menstruated within the past 2 months), perimenopause (women who have menstruated within the past 3-12 months, but when the menstrual cycle becomes irregular) and postmenopause (women without menstruation for 12 consecutive months) (WHO 1981, 1996). Therefore, menopause is the end of the female

reproductive life, after which women cannot establish any pregnancy.

The earliest known experiment in reproductive biology was recorded in 1787 and was carried out in London by the eminent surgeon John Hunter (Biggers et al., 1962) who compared litter sizes of hemi-ovariectomised (removal of one ovary) sows with normal breeds. He observed that the litters produced by the hemi-ovariectomised sows were smaller than those of the normal breed. A similar experiment was replicated after two hundred years by Anne McLaren and John Biggers at the Royal Veterinary College, London, on hemi-ovariectomised mice and normal mice in the laboratory (Biggers et al., 1962). They compared the reproductive capacity of both groups of mice for several years. Like Hunter, this study revealed a smaller litter size among the hemi-ovariectomised mice which ceased producing litters earlier than normal mice (Biggers et al., 1962). Studies in assisted reproduction found a higher total number of follicles and higher E2 levels in women with two ovaries than women with one ovary (Alper et al., 1985). Khalif et al. (1992) reported that women with one ovary had increased basal FSH levels and a poorer outcome in assisted reproductive treatment compared to women with two ovaries. Therefore, it is evident that the ovary is limited in

producing ova (ovarian reserve) and this affects the process of fertility.

Concern about ovarian reserve and reproductive ageing grew in the early 1970s given the low fecundity of women in their thirties resulting from delayed childbearing caused by career and other priorities (Menken et al., 1986). This social issue has increased in recent years. Assisted reproductive technologies (ARTs) have developed to resolve the problem of infertility whether among younger or older women. In 1978, with the birth of Louise Brown, in-vitro fertilisation (IVF) has proven to be an efficient way of treating unexplained infertility and, this year, won Dr Robert Edwards a Nobel Prize for medicine. Although age and fertility are negatively correlated, age is not always a good marker for predicting reproductive success. Some women in their early thirties fail to get pregnant without any obvious pathology. On the other hand, some women conceive in their forties. The difference lies in ovarian reserve and numbers of eggs remaining in the ovary.

The reproductive "switch off" at menopause occurs due to depletion of follicles (exhaustion of ovarian reserve). Forabosco and Sforza (2007) suggested two functional pools of follicles: primordial ("resting") and a growing follicular pool, with the resting pool representing the ovarian reserve from which follicles

are recruited to the growing pool for maturation. Therefore, ovarian reserve is defined as the size of the ovarian follicle pool, and the quality of oocytes therein declines with increasing age and results in a decrease in women's reproductive function (Bentley and Muttukrishna, 2007). The size of the pool is established at an early stage of life, as every female is born with certain numbers of primordial follicles and no new ova are formed after birth.

This view of a finite primordial follicle pool has been challenged by Johnson et al. (2004, 2005), who claimed that germ line stem cells can repopulate a postnatal ovary and regenerate the primordial follicle pool, and this observation was supported by Bukovsky et al. (2004, 2005). However, these suggestions have been vigorously criticised as there has been no independent confirmation or replication of the proposed study (Gosden, 2004; Greenfield and Flaw, 2004; Teffler, 2004), while an illustrative new study shows that oocyte regeneration is not possible in adult mammals by circulating germ cells (Eggan et al., 2006).

Several million germ cells develop by 20 weeks of intra-uterine life (Themmen, 2005; Leidy 2006) and, consequently, a pool of primordial follicle is established, from which a cohort of follicles starts to grow commencing during intra-uterine life. These growing follicles progress to the antral stage, at which point they eventually undergo atresia (degeneration and subsequent

resorption of the immature ovarian follicle). About 2 million primordial follicles are present in the ovary at birth. Primordial follicles continuously enter the growing follicle pool (follicular "recruitment") and develop from pre-granulosa cells to granulosa cells, a process which continues throughout life until the follicular pool is exhausted. In pre-pubescent girls, all the recruited follicles that start developing are lost through the process of atresia. Following activation of the HPG axis at puberty, some follicles are "rescued" by FSH – a process of "selection" and can ovulate under the control of luteinising hormone (LH).

At puberty, there are about 300,000 to 500,000 primordial follicles and a pool of follicles continues to grow in each cycle. Under control of FSH and LH, usually only one follicle is designated to become the dominant follicle which ultimately becomes mature and releases its oocyte for fertilization under the influence of LH. In the long run, only a small percentage of ova have the chance to mature and ovulate and eventually have a chance to be fertilised during a women's reproductive life. Therefore, reproductive function in women depends on how many eggs are in the ovary that are able to grow and ovulate. This reserve can be predicted by measuring the number of follicles that start to develop using the proxy of reproductive hormone levels – FSH, inhibin B and

AMH, on days 2-4 of the menstrual cycle (Muttukrishna et al., 2001).

However, several other studies have suggested that during the early follicular phase, inhibin B starts to rise and peaks at around days 5 to 6 in normal cycling women, while AMH levels show no remarkable fluctuation throughout the cycle (Muttukrishna et al., 2004, Bentley and Muttukrishna, 2007; Groom et al., 1996). On average, 85 days or three menstrual cycles are needed for the growth of ovarian follicles from an early pre-antral stage to the ovulatory stage (Bentley and Muttukrishna, 2007). Therefore, an ovary of a woman of reproductive age contains all stages of follicles i.e. primordial to pre- and postovulatory (corpus luteum and albicans) follicles. As the follicular pool is established during early life and is continuously depleted throughout reproductive life, female ovarian function declines due to depletion of ovarian reserve. The exhaustion of ovarian reserve results in termination of reproductive life with cessation of menstruation.

Age at menopause varies quite considerably, such as, between 49-51 years in women from industrialised countries (van Noord et al. 1997) and between 43-47 years in women in developing countries (Wasti et al. 1993). Increased FSH and decreased E2 and inhibin B (Muttukrishna et al. 2000) are

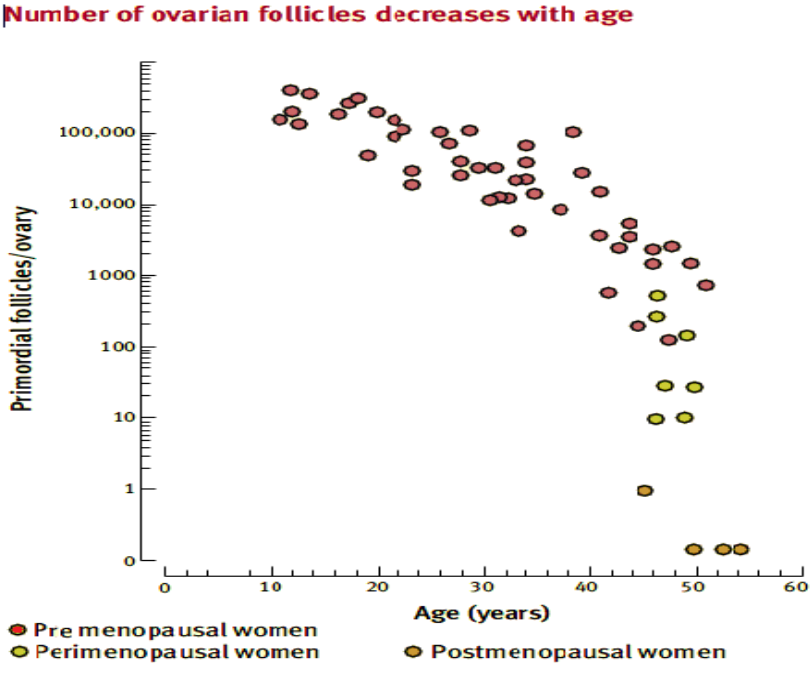
features of ovarian depletion. As E2 and inhibin B both have an inhibiting effect on FSH, a rise in FSH levels with ageing is related to a decline in the inhibitory effect of the former two hormones. This changing pattern of hormones indicates reproductive ageing. However, menopause itself is not the result of changes in the HPG hormonal feedback loop. It is rather a consequence of exhaustion of the ovarian follicular pool (reserve).

As the ovarian follicles are fixed in number from foetal life, follicular numbers decline with age (Figure 1.3) through the process of either atresia or development towards ovulation, (Baker, 1963). Several researchers have tried to construct models for predicting follicular count (ovarian reserve) at a given time. Faddy (1992) suggested that the decline of follicles in the human ovary does not conform to a simple exponential model. After analysing log transformed data from Block (1952 and 1953), Gougeon (1994) and Richardson et al. (1987), Faddy (2000) proposed that the decline of follicle numbers with age is bi-exponential (bi-phasic), indicating that follicular depletion is accelerated at a certain age (38 years) or a certain point of ovarian reserve (at about 25,000 follicles). It has been suggested that regularly menstruating women have a higher primordial follicle count than irregularly menstruating women (Richardson et al., 1987) and that the duration of the menopausal transition

(interval between beginning of menstrual irregularity and menopause) is, on average, 6 years (den Tonkelaar et al., 1998).

Leidy et al. (1998) argued against a bi-phasic model for follicular depletion and suggested that the results reported by Faddy (1992) were not consistent with the data. A possible explanation was due to logarithmic transformation of the original data. They also argue that Faddy (1983) had excluded some data from his analyses. This argument was further extended by McDonough (1999) who suggested that the data was misinterpreted as the bi-exponential regression arises from the log-transformation of the follicular count. Finally, Leidy et al. (1998) suggested that the depletion of follicles is constant, and there is no accelerated depletion in later life.

Figure 1.3 Follicular depletion with age (adapted from Burger, 2006)



1.5 Changes in reproductive hormones with reproductive ageing

The ovary gradually loses its functional capacity (ovulatory efficiency and hormone production) with increasing age. Variability in menstrual cycle length is common to woman at both ends of their reproductive life (following menarche and preceding menopause). Although menstrual irregularities with unusually long and short cycle lengths appear during the menopausal transition, cycle length gradually shortens with increasing age until the menopausal transition (Treloar, 1967). A longer menstrual cycle in young women suggests a delayed follicular maturation, while a shorter cycle in the premenopausal women indicates a short follicular phase. The changes in functional capacity and menstrual cycle regularity are mirrored in the accompanying hormonal changes.

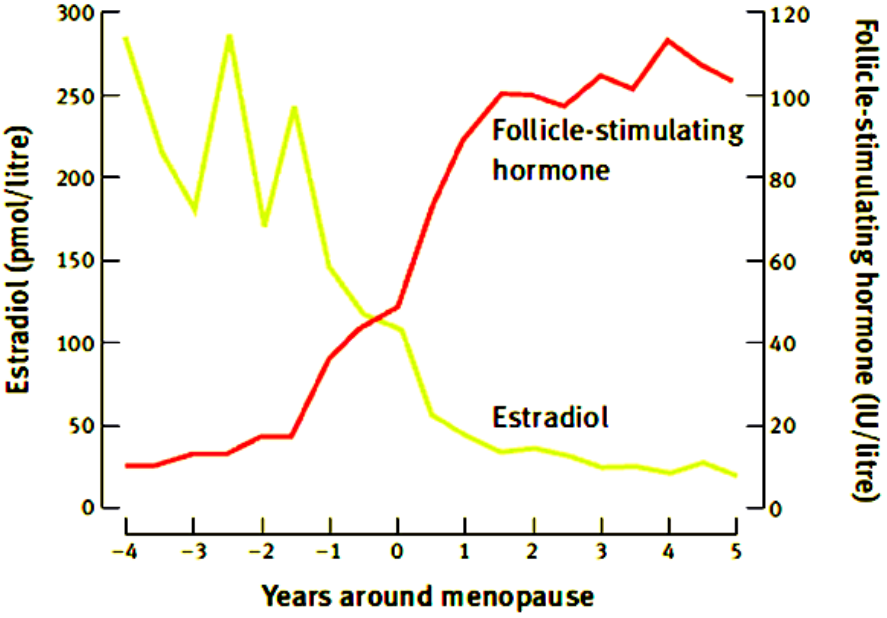
Women in their forties experience a gradual elevation in serum FSH levels with increasing age during the follicular phase, even though they might have regular menstrual cycles (Burger et al., 2000), while levels of circulating E2 increase slightly during this time, but serum inhibin B levels decline. The Melbourne Womens' Midlife Health Project (MWMHP) observed that inhibin B indices start to decline and FSH indices start to rise during the menopausal transition when the decline in follicle numbers

reaches a critical threshold. In post-menopausal women, there are markedly raised FSH and low E2 levels with undetectable inhibin B and AMH (Burger et al., 2007). The explanation for the increased FSH level with ageing is reduced control of negative feedback by the decreased inhibin B levels. It is suggested that menopause results from the loss of follicular activity, but is not due to complete exhaustion of ovarian reserve. Rather the follicular pool is reduced below a critical threshold (Leidy et al., 2006). Therefore, with the disappearance of follicles, the source of ovarian estrogens and inhibin B is lost and there is insufficient E2 to sustain the menstrual cycle.

One of the features of the menopausal transition is menstrual irregularity that begins before the cessation of menses and is associated with decreases in levels of early follicular-phase inhibin B (Burger, 2006). FSH levels begin to increase further from premenopausal levels. Women whose menses occurs at intervals of more than 3 months (late perimenopause) exhibit increased FSH, with a substantial decrease in circulating E2 and inhibin B. Serum FSH levels at the time of final menses are 10-15 fold higher than that of the follicular phase at reproductive age (Burger et al., 1999), while serum E2 levels are about 50% less than those of the follicular phase during reproductive life. E2 levels continue to decline after final menses to reach their lowest point

2–3 years later, which is about 90% or more lower than those of reproductive life (Burger et al., 2006). Therefore, the menopausal transition is a time of marked hormonal instability.

Figure 1.4 Geometric mean levels of follicle-stimulating hormone and E2 around the time of the final menses (Source: Burger, 2006)



Repetitive hormonal sampling in an individual woman may show various patterns, with high or low FSH, E2 and inhibin B depending on the characteristics of that particular cycle. In a population of women, the major changes around the final menses are a progressive increase in FSH and a progressive decrease in E2 (Figure 1.4). However, FSH levels are not significantly raised until the menstrual cycle has become irregular, but serum AMH changes comparatively earlier in the sequence of ovarian ageing. In one study, de Vet et al. (2002) found that AMH levels decline

significantly with age among young women when they were tested at three year intervals, although serum FSH and inhibin B were not changed during this interval.

1.6 Assessment of ovarian reserve

Although the ovarian reserve that represents the size of the ovarian follicle pool is difficult to assess, the number of actual primordial follicles that are left in the ovary is also an important parameter for ovarian reserve (te Velde and Pearson, 2002). However, the size of the primordial follicle stock in women is difficult to measure directly, although it appears that the number of growing follicles is correlated to the size of the primordial follicle reserve from which they are recruited (Scheffer et al., 1999). Therefore, a marker that reflects all follicles that have made the transition from the primordial follicle pool to the growing pool may be a good indirect marker of quantitative aspects of ovarian reserve.

In earlier demographic studies, age was evaluated as a predictor of women's fecundity (Nader and Berowitz, 1991). Lipson and Ellison (1996) suggested that the success of conception is directly related to levels of E2. On the other hand, several authors (Scott et al., 1989; Lee et al., 1988) argued that conception is not related to E2 levels and there is no significant

difference in these levels in regularly menstruating women either in their twenties or fifties. However, each of these studies followed a different methodology e.g. Lipson and Ellison tested daily saliva samples over the cycle, while others tested single blood samples over the cycle.

Despite the diagnosis of the menopausal transition or menopause, ovarian reserve assessment is crucial for the success of potential assisted reproductive technologies (ART) such as in vitro fertilisation (IVF). The assessment of ovarian reserve in ART is essential in order to identify the potential of women to become pregnant after IVF treatment. Therefore several screening procedures have been used to assess ovarian reserve.

Follicular phase length reduces gradually with increasing age (Sherman et al., 1975), which is expressed by relatively high E2 levels on cycle day 3 due to early follicular recruitment (Licciardi et al., 1995). In one ART programme, it was observed that there is a negative correlation between levels of E2 on cycle day 3 and the chance of conceiving (Smotrich et al., 1995). However, studies suggest that serum E2 levels on cycle day 3 alone cannot predict ovarian reserve (Mukherjee et al., 1996; Scott et al., 1989).

The early follicular phase (cycle day 3) FSH rise was suggested as a marker of reduced ovarian function (Scott et al.

1989). It has been suggested that the chances of getting pregnant are less when FSH levels are elevated (≥ 20 mU/ml, Martin et al., 1996). Although early follicular phase FSH levels were considered as standard marker of reduced ovarian function, or diminished responsiveness of the ovary to ovulation induction in clinical setup, ovarian reserve assessment is crucial for the diagnosis of the menopausal transition or menopause and also for the success of potential ART and IVF. The assessment of ovarian reserve in ART is essential in order to identify the potential of women to become pregnant after treatment. Later, Mukherjee et al. (1996) suggested that the FSH:LH ratio on day 3 is a more useful predictor for IVF outcome in women than that of day 3 FSH measurement alone.

Several other tests have been used to evaluate ovarian reserve of women using serum E2 and FSH levels in clinical settings. Navot et al. (1987) introduced the clomiphene citrate challenge test (a selective oestrogen receptor modulator mainly used for induction of ovulation) to predict ovarian reserve. Woman are given clomiphene citrate (100mg) during cycle days 5 to 9, and FSH level is measured on cycle days 3 and 10 to predict ovarian reserve. A high FSH level in response to this challenge indicates that there is not enough E2 or inhibin B to suppress FSH, which ultimately indicates a lower number of follicles or ovarian

reserve. Therefore, this test can differentiate between normal or reduced ovarian reserve.

In addition to FSH and E2, assessing serum levels of inhibin B in the early follicular phase has now been used to predict ovarian reserve. As inhibin B and E2 are produced by early antral follicles in response to FSH and are involved in the feedback loop, serum levels of all three hormones are interdependent (Burger et al. 1999) with negatively correlated serum FSH and inhibin B levels (Muttukrishna et al., 2000). Klein et al. (1996) reported that older women (>38 years) with elevated early follicular phase FSH levels had significantly lower inhibin B levels compared to younger women (20-25 years). Therefore, the secondary rise in pituitary FSH production is due to a decreased production of inhibin B by a reduced number of growing follicles in the ovary.

As AMH reflects the size of the growing follicle pool, serum levels on days 2-4 of the cycle can predict the ovarian reserve in that particular cycle (Durlinger et al. 2002a; Hudson et al. 1990; Lee et al. 1996). Moreover, AMH is not controlled by gonadotropins and, therefore, has been used as a marker for ovarian reserve in ART recently. Age-related follicular attrition causes decreased serum AMH levels (de Vet et al., 2002) and reduced follicular count (Reuss et al., 1996) with advancing age. In a group of older women (>38 years) presenting for IVF

treatment, Muttukrishna et al. (2004) found a ten-fold lower level of serum AMH in women with non-viable cycles in response to gonadotropin stimulation compared to women with viable cycles. The study also revealed that serum AMH levels had the most significant positive correlation with the number of eggs retrieved from women among the three hormones examined. In an IVF setting, cycle viability is explained by ovarian responsiveness which is defined as the number of oocytes retrieved (viable cycle), or as cancellation of procedure due to impaired or absent follicular growth (non-viable) (Visser et al, 2006). Serum AMH and antral follicular count (AFC) were found to be significantly higher among the normal responders to IVF compared to poor responders, while serum AMH levels strongly correlated with AFC, the number of follicle retrieved, age, inhibin B and FSH levels (van Rooij et al. 2002).

Several other studies have suggested that the correlation between serum AMH levels and retrieved oocytes in women undergoing IVF treatment, and serum AMH and AFC are equally important predictors (Seifer et al., 2002). Therefore, serum AMH measurements in the early follicular phase appear to be a better predictor of the number of early antral follicles than other hormonal markers. In one study, van Rooij et al. (2004, 2005) reported that serum levels of AMH gave the highest accuracy

(0.87) to predict the occurrence of the menopausal transition within 4 years when several markers of ageing were measured in normal women at 4-year intervals. Inclusion of serum inhibin B levels and age in the multivariate model improved the accuracy to predict the transition further to 0.92 (van Rooij et al. 2004, 2005). Overall these results indicate that serum AMH levels are a good marker of ovarian reserve.

Although research suggests that AFC can predict ovarian response to successful IVF stimulation (Verhagen et al., 2008) and is the first choice for assessing the diminished ovarian reserve, further research with AMH suggested that AMH has the potential to predict ovarian reserve similar to AFC in an IVF treatment setting (La Marca et al., 2007; Broer et al., 2008; Elgindy et al., 2008; Kwee et al., 2008). As AMH is produced in the pre-antral stages, AMH levels represent the cohort of primordial follicles (Kevenaar et al., 2006).

Further study on intra- and inter- individual variations of AMH and AFC were examined and suggested that there are no significant differences between AMH levels and AFCs (van Disseldrop et al., 2010). Other studies (Scheffer et al.,1999; Hansen et al., 2003; Bancsi et al., 2004; Elter et al., 2005; Mcllveen et al., 2006; Streuli et al., 2008) on inter-cycle variability also suggest that AMH does not vary significantly between the

cycles. Although AFC and AMH apparently do not differ between and within cycles (Hehenkamp et al., 2006; Wunder et al., 2008;Streuli et al., 2009), AMH displays less intra-individual fluctuation than AFC both within and between cycles (La Marca et al., 2007; van disseldrop et al., 2010). This suggests AMH to be the better cycle-independent parameter to assess ovarian reserve.

Research suggests that there are several advantages of AMH over AFC as it is a laboratory test (Broekmans et al., 2009) whereas AFC is a biophysical test using ultrasound technology. Moreover, most studies consider AMH to be cycle independent (Hehenkamp et al., 2006; La Marca et al., 2006; Tsepelidis et al., 2007). AFC might be more prone to observer bias and show more variance between cycles in patients (Scheffer et al., 2002; Fanchin et al., 2005; van Rooij et al., 2005). Therefore, AMH levels could be an ideal ovarian reserve test that would represent one, preferably cycle independent, measurement to represent ovarian reserve status.

Furthermore, the use of AMH as a marker of ovarian reserve was investigated in young women after treatment for cancer in childhood (Bath et al., 2003). Chemotherapy and radiotherapy treatment have adverse effects on the ovary in particular, resulting in loss of primordial follicles, while young cancer survivors suffer a partial loss of ovarian reserve. The partial loss of

ovarian reserve is reflected by decreased AMH, increased FSH levels and decreased ovarian volume. The decrease in serum AMH levels in these patients further supports the use of serum AMH levels as an early predictor of ovarian reserve.

This chapter has provided background to understanding reproductive physiology, ovarian and reproductive ageing as a context for the study of ageing in Bangladeshi women. The next chapter presents a background to a more ecological and evolutionary perspective on reproductive function that also provides a broader context in which to place this study of migrants Bangladeshi women.

CHAPTER 2

LIFE HISTORY THEORY, EARLY LIFE DEVELOPMENT AND REPRODUCTIVE FUNCTION

2.1 Human development and female reproduction

The human life cycle stands in sharp contrast to other species of social mammals even other primates. The pattern of human postnatal growth can be characterised by several stages: infancy, childhood, juvenile, adolescence, adulthood (reproductive period) and a prolonged postmenopausal life (Bogin, 1993). In contrast, social mammals have three basic stages of postnatal development: infancy, juvenile and adult with some species having brief periods of post-reproductive life (Bogin, 1993, 1990). Changes in trophic and reproductive behaviour are associated with each stage. The stages of the life cycle can be defined by biological characteristics like rate of growth and sexual maturity. Usually, in mammals, progression from infancy to adulthood is continuous, while highly social mammals and primates delay sexual maturity by inserting a period of juvenile growth between infancy and adulthood (Nishida et al.,1990). In the human lifecycle, slow growth pattern during early life result in the dependent period of infancy is followed by further period of dependence during the stage of childhood prior to the independent

juvenile stage. There is also insertion of an adolescent stage between the juvenile and adult matured stage that is marked by some visible sign of sexual maturation like secondary sex character like development of breast. During adolescence a rapid acceleration of growth occurs, and is referred to as the "adolescent growth spurt". At this point, individuals reach adult stature and achieve full reproductive maturity. Therefore, the prolonged period of childhood dependency, the slower growth pattern during early life and the delayed growth spurt during adolescent stage are responsible for the delayed reproductive maturity (Bogin and Smith, 1996).

In addition to childhood and adolescence, there is another unusual aspect of human life history - menopause, which occurs as cessation of normal menstrual cycle due to loss of ovarian function subsequent to the depletion of ovarian follicle. From a comparative primate and evolutionary perspective, menopause is a virtually universal and occurs at about 50 years of age; nonhuman primates living in the wild do not share the uniqueness of a menopause (Pavelka and Fedigan, 1991). Some studies suggest that no wild living species except perhaps the short-finned pilot whale (Austed, 1994), and African elephant (Austad, 1997, Finch 1990) exhibit reproductive cessation at later life., Female primates studied in captivity, including langurs, baboons,

rhesus macaques, pigtailed macaques and chimpanzees usually continue oestrus cycles until death, although there is a fertility decline with age (Graham et al., 1979; Gould et al., 1981; Nishida et al, 1990; Pavelka and Fedigan, 1994;). This decline can be best interpreted as a normal part of ageing. In contrast, the human female reproductive system is shut down well before other system of the body, which usually have a more gradual decline towards senescence. Furthermore, women can live for decades after oocyte depletion (menopause), but other female primates die before or just after oocyte depletion. Therefore, menopause is a unique and universal female reproductive phenomenon for human.

Although not the focus of this dissertation, a short summary of theories surrounding the menopause will be useful. An evolutionary perspective on menopause has led to arguments whether it is an evolved phenomenon or an accidental by product of the extended lifespan in humans. Proponents of the former present some version of parental investment theory. Menopause, specifically, may have evolved as a result of the rapid encephalisation of human in combinations with acquisition of bipedal movement (Peccei, 2001, Williams, 1957). Evolution of bipedal movement resulted in pelvic alterations that made delivery more dangerous and difficult. Therefore, with increasing age, the mortality risk and energetic requirements of pregnancy, delivery

and lactation became too great to risk for women. Furthermore, increased brain size resulted in a more altricial infant (helpless) with an extended period of parental care. The extreme altriciality of human infants requires a large parental investment. Thus, the Good Mother Hypothesis (Alexander, 1974; Nesse and Williams, 1996; Sherman, 1998) states that there is a trade-off between increasing allelic contribution to the population (having more children) and ensuring the survival of present children. The Grandmother Hypothesis (Blurton Jones et al., 2002; Gibbons, 1997; Hawkes et al., 1998) is an extension of this theory, but it more directly addresses the post-menopausal period. The suggestion of these theories is that women can maximise their reproductive success in middle age, if they cease reproduction of new children and concentrate instead on investing in her lastborn child or grandchildren.

The "Grandmother Hypothesis" agrees with the general risk of pregnancy at advanced ages, but also addresses the contribution of the grandmother to her grandchildren in assuring her own personal "fitness," that is, a re-allocation of her reproductive effort to care for children already born, as opposed to production of offspring unlikely to survive maternal death. Although this reproductive strategy is thought to unique to humans and possibly some non-human primates, both elephants

and pilot whales also show similar social structure that of human and have an extended period of post-reproductive lifespan. It is plausible that in some higher vertebrates, ”grandmothering” is useful where extensive caretaking and energy investment is required for dependent offspring. Another aspect of the Grandmother Hypothesis suggests that senescence is actively selected so that parents remove themselves from the competition with their off spring (Lancaster and king, 1992). Thus menopause is only an early step along the way to withdrawal from competition.

Several authors (Washburn, 1981: Weiss, 1981) have rejected the view of menopause being an evolved phenomenon. It has been suggested that menopause is simply a non-adaptive by-product of increased longevity in humans, since it seems contrary to maximizing Darwinian fitness (Blurton Jones et al., 2002). That is, rapid advances in medicine, nutrition and health have allowed people to live much longer than they have in the past, so there was likely little to no post-reproductive life in the past. It is believed that not enough time has passed for an evolutionary effect and menopause is little more than a side effect of increased lifespan (Wu et. al., 2005). However, there is neither any consensus “how menopause evolves” nor any agreement about whether has adaptive value.

2.2 Life history, adaptation and plasticity

From an evolutionary perspective, features of physiological and behavioural patterns in terms of growth, maintenance and reproductive characteristics during the life course are explained by Life History Theory (LHT). LHT posits that available resources are allocated to the competing components of growth, maintenance and reproduction (Williams 1957). Since resources may be limited (Gadgil and Bossert 1970; Ellison 1990), trade-offs are essential for optimum allocations in the regulation of the life history model and play a central role in LHT. The degree of trade-off is dependent on resource availability and, where populations have limited resources, there will be greater trade-offs.

Trade-offs mainly occur between energy expended on growth, factors influencing mortality (maintenance) and reproduction (Stearns 1992). Many characteristics of life history have opposing effects on mortality and fertility, and therefore on present and future fitness (Hill and Kaplan, 1999). A typical component of life history models is a trade-off between juvenile developmental time and size at maturity (Roff, 1992; Stearns, 1992).

Developmental or phenotypic plasticity is a powerful means of flexible adaptation for an individual, which allows organisms to

modify their biological settings across the life-course. Plasticity allows an individual to use environmental cues to optimise its life-course strategy. The ability to respond to environmental changes is established through altering physiological homeostatic control processes (Waddington, 1956) and/or structure during development.

2.3 Foetal programming, Predictive Adaptive Responses and Environmental Mismatch

It is well documented that growth, maintenance and reproduction are influenced by environmental, nutritional and hormonal conditions during early life (Metcalfe and Monaghan, 2001). In 1986, from the finding of the retrospective data from 1921 to 1978 for mortality rates from stroke and cardiovascular diseases (CVD) in the UK, Barker and colleagues concluded that measures of poor health in mothers were important causal factors for the risk of stroke in their children during adult life. They developed the "Early or Foetal Origins of Adult Disease" or "Foetal Origin Theory" and suggested that environmental factors, such as nutrition, influence early life to programme the risk of early onset CVD and metabolic disorders in adult life (Barker et al., 1989; Barker, 1998; Osmond et al., 1993). Their particular focus has been on uterine and early post-natal life.

Following the rise of the "foetal programming" concept, a series of global epidemiological studies have been carried out to further this work. Although studies initially examined only the influence of pre- and post-natal growth on risk of developing CVD in adult life, later studies examined the relationship of the early developmental environment on risk of other metabolic disorders such as glucose tolerance, type 2 diabetes, insulin resistance, and obesity in adult life (McMillen and Robinson, 2005).

Following from this work, Hales and Barker (1992) proposed a "thrifty phenotype" hypothesis that suggested there is an adaptive response to poor foetal environments for maintaining the effective function of key organs at the cost of other organs or physiological functions, which would lead to an altered postnatal metabolism. In response to under-nutrition, placental dysfunction, or other adverse influence, the foetus can change its trajectory of development and can slow its growth (Barker, 1998). Babies born with low birth weight have a reduced functional capacity or fewer cells in key organs that affect metabolic processes (Bateson et al., 2004). For example, people who have low birth weight are resistant to the effects of insulin in moving glucose out of the blood into the tissues. This is in order to protect an uninterrupted glucose supply to the brain during foetal life. Infants with this

adaptive response develop a characteristic phenotype that persists into adult life (Armitage et al., 2004).

Most of the previous studies researched the impact of the intrauterine environmental condition on adult health and disease. However, a study by Osmond et al., (1993) observed that the highest incidence of death from CVD is associated with low birth weight and low weight at age one. Other studies undertaken to understand developmental plasticity have suggested that nutritional, hormonal and other aspects of the prenatal *and infant environment* have effects on physiology and metabolism that persist into adult life (Osmond et al, 1993; Barker, 1994; Bateson et al., 2004, Eriksson et al., 2004; Costello et al., 2007, Ellison and Jasienska, 2007). Rapid postnatal growth particularly following restricted foetal growth as catch-up growth, may exacerbate the physiological changes that lead to CVD and adult metabolic disease (Lucas et al. 1999, Metcalfe & Monaghan 2001, Cameron 2007). However, almost no studies have concentrated on development beyond infancy.

Human development may generate specific patterns of development through present environmental cues which prepare the individual for the possible future environment. If the future environment is not as predicted (i.e., it is mismatched) then the individual may be affected adversely (Bateson, 2001). For

example, a lower birth weight baby who subsequently grows up in an affluent environment, is at increased risk of developing CVD, type 2 diabetes and hypertension (Barker, 1998; Godfrey et al., 1997; Gluckman and Hanson, 2004), while an individual born heavier and subsequently living in an affluent environment is at less risk (Eriksson et al., 1999). Therefore, it is evidenced that a substantial mismatch occurs when the adulthood environment differs significantly from the developmental environment. This is explained as the "Environmental Mismatch" hypothesis (Barker, 1998), and suggests an inappropriate prediction during early life leads to greater risk of disease in the future.

To explain this mismatch phenomenon, a phenotypic response is induced during development in response to environmental cues in order for an individual to obtain an adaptive advantage that will persist during adult life. This phenotypic response is termed the "Predictive Adaptive Response" (PAR) (Gluckman et al., 2005). PARs will be adaptive if the prediction is correct, and will be maladaptive if it is not. The latter usually occurs in industrialised, urban societies where migration frequently occurs. The phenotype is beneficial when there is an appropriate prediction, but relative fitness of the phenotype to the predicted environment depends on the degree of environmental

change relative to developmental period (Jablonka et al., 1995; Moran, 1992; Sultan and Spencer, 2002).

Most of the studies related to foetal programming or adaptive responses were focused on metabolic syndrome, CVDs and type 2 diabetes (Barker et al., 1989; Barker, 1998; Osmond et al., 1993), while very little work has been done on reproductive function. Moreover, studies were mostly focused on the uterine environment. There is beginning to be a better appreciation of the postnatal period especially where there is very rapid growth following low birth weight. There are some studies that have looked also at very early childhood and particularly where infants are born with low birth weight and then grow quickly (Lucas et al. 1999; Metcalfe and Monaghan 2001; Cameron 2007). Few studies have looked at the early childhood period on its own merits (Hardy and Kuh 2005 & 2002; Nunez et al. 2007).

Some studies have been carried out to examine the impact of childhood developmental condition on adult life, particularly reproductive maturity and reproductive function. For example, childhood weight gain was suggested as an important indicator for age at menarche. In one study on age at menarche according to birth weight and weight at the age of seven, a national sample of British girls yielded data to suggest that girls who had lower birth weights but gained weight rapidly in childhood had an earlier age

at menarche. On the other hand, those who had higher birth weights but lower weights at the age of seven had a later age at menopause (Barker, 1998). Another study on British women suggested that women with lower weight at the age of two, or a stressful home environment in a lower socio-economic family during childhood, had an earlier age at menopause (Hardy & Kuh 2005; Hardy & Kuh 2002). Therefore, childhood constraints not only include nutritional deprivation but also overall socio-economic conditions.

2.4 Reproductive Function

As previously mentioned, LHT is based on the well-defined assumptions of energy allocation decisions between competing physiological needs of growth, maintenance and reproduction. It is crucial for understanding interactions between reproductive function and other aspects of physiology. An evolutionary and life history perspective to understand reproductive physiology goes beyond biology, and into the field of health research. For example, physiological responses to immune challenges such as infections can be considered as adaptive rather than pathological (Williams and Nesse, 1991). Therefore, many health issues related to female reproductive function are likely to have evolved as a result of life history tradeoffs.

A significant range of variation exists in female reproductive function and reproductive hormone indices. Central sources of variation include chronic energetic status and age, both within and between populations. A life history model can provide useful insights into this relationship. The timing of key life history events, particularly menarche and menopause are central to lifetime reproductive function.

Female reproductive maturity is generally indicated by first menses and age at menarche is one of the milestones of reproductive function. The timing of this life history event is crucial since variation directly affects total reproductive life span and reflects a shift in energetic investment from growth to reproductive effort (Stearns, 1992). It is well established that there is wide variation in age at menarche between developed and developing countries (Tanner 1973). For example, studies suggested that the median age at menarche of girls of developed countries like the US is 12.43 years (Chumlea et al., 2003) and for UK girls is 12.9 years (Whincup et al., 2001). In contrast, average age at menarche in developing countries like the Dominican Republic is 13.1 years (Mencebo et al., 1990), in South Indian schoolgirl is 13.5 years (Bai and Vijayalakshmi, 1978) and in Tianjin, China is 14.4 years (Wang et al., 1992).

A common explanation to address this variation was the Frisch Hypothesis (FH), which suggested that sufficient body mass, specifically adiposity, was crucial for triggering first menses (Frisch, 1971 & 1974; Frisch et al., 1971). Although the role of adiposity on menarche can be explained rationally in view of the metabolic cost of female reproductive function, there is not much available evidence to support this hypothesis. An alternative hypothesis suggests a better predictor for menarche is skeletal growth (Matkovic et al., 1997) and particularly bi-iliac width (Ellison, 1982) because a rapid acceleration in the growth of skeletal tissues occurs during adolescence, termed the adolescent growth spurt that serves as a signal of maturation (Bogin and Smith, 1996).

Variability in age of menopause between populations has been reported in several studies particularly between developed (MacMahon and Worcester, 1966; Stanford et al., 1987; Whelan et al., 1990; McKinlay et al., 1992; Parazzini et al., 1992; Rebato, 1988; Prado and Canto, 1999; Luoto et al., 1994).and developing countries (Beyene and Martin, 2001; Garrido-Latorre et al., 1996; Wood et al., 1985; Sarin et al., 1985; Randhawa et al., 1987; Wasti et al., 1993; Goodman et al., 1985). Although explanation for this variation includes genetic factors, a significant role is also played by ecological factors that affect biological processes

(Lummaa and Clutton-Brock, 2002). For example, Cresswell et al., (1997) found an earlier menopause in women born with low birth weights and low weight gain in early childhood.

Energetic factors influence ovarian function, which is highly dependent on energy balance and expenditure. In many traditional populations (e.g., Benefice 1996; Panter-Brick 1993; Bentley et al, 1998), nutritional scarcity and high energy expenditure particularly in demanding seasons cause lower energy availability. To deal with this lower energy availability, women evolved adaptive mechanisms through suppression of reproductive function, which can be explained by life history theory of energy allocation and trade-offs between competing compartments of growth, reproduction and maintenance. It is suggested that reduced levels of ovarian hormones occur in energetically stressed environments that slow reproductive success in persistently energy-deprived populations (Ellison, 2001). Vitzthum, Spielvogel & Thornburg (2004) found that progesterone levels of rural Bolivian women were significantly lower than Chicago women in both the follicular and luteal phases. The adaptive response of reproductive suppression was suggested to be due to negative energy balance in Bolivia.

Some studies have shown that variation in ovarian function is dependent on energy expenditure alone. For example,

suppression of ovarian steroids is associated with vigorous aerobic exercise even though energy balance is maintained by increased intake of calories (Bullen et al., 1985). Moreover, lower progesterone levels were observed in hardworking farm women in Poland during the physically demanding season of agricultural work, which suggested that suppression of this hormone is not related to energy balance but rather to increased energy expenditure (Jasienska, 1996). Therefore, low energy input or high energy output both cause alteration of energetic conditions which eventually leads to suppressed reproductive function (Jasienska & Ellison, 2004).

Variability in hormonal levels among populations living in contrasting ecological settings with variable energy balance has also been suggested by several studies. A study of Lese horticulturists of Zaire's Ituri forest showed lower progesterone in women when they lost weight during the hunger season, but levels reversed after the harvest (Bailey et al., 1988; Bentley et al. 1998; Ellison et al., 1989). Similarly, among Nepalese horticulturists, seasonal nutritional deprivation with increased expenditure due to hard work resulted in low ovarian steroid hormones (Panter-Brick, 1992; Panter-Brick & Ellison, 1994). However, ovarian steroid levels during even good nutritional

periods are still lower compared to women in developed countries (Ellison 1993).

Data on progesterone levels of adult women from five populations (Boston women, rural farmers in Poland, Quechua Indians in Bolivia, Lese horticulturalists in Zaire and Tamang agro-pastoralists of Nepal) living under variable ecological settings with different energetic conditions were compared. There was significant variability in age specific ovarian steroid levels across these populations, but with similar patterns of variation of progesterone across age groups (Ellison et al. 1993). Moreover, menarcheal age in these groups showed a negative relationship with progesterone levels and was found to be variable across the populations. The youngest mean menarcheal age and highest progesterone levels were in Boston women, while the oldest menarcheal age and lowest progesterone levels were among the Nepali women. There are two possible mechanisms that have been suggested to explain the consistent difference in ovarian function between populations. These are either acute conditions that regulate ovarian function or chronic developmental effects, however, actual mechanism has not yet been clearly identified.

Therefore, environmental factors have an impact on endogenous steroid production in both males and females (Ellison et al., 1993), but this is also influenced by constitutional (genetic) and

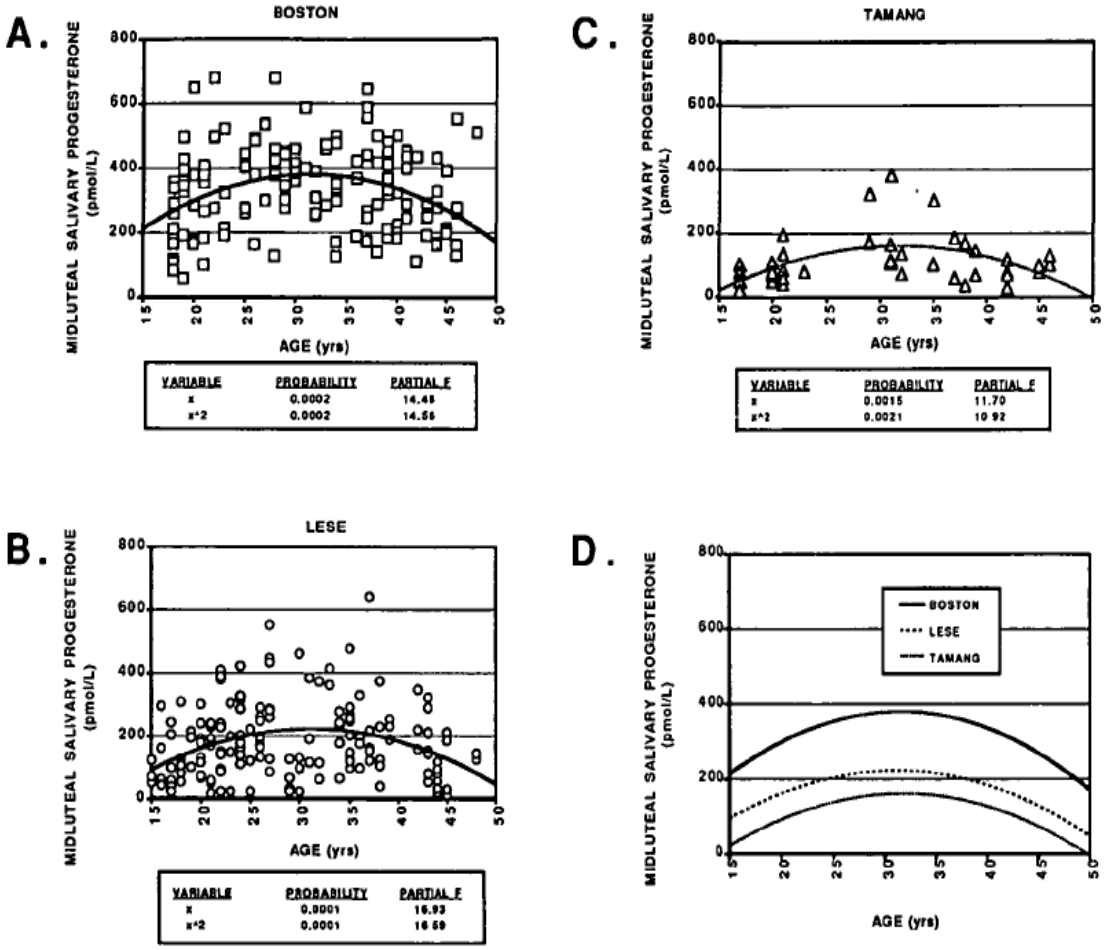
behavioural factors (Ellison, 1999). As reproductive function in both males and females depends on the supply of energy (Panter-Brick, 1999), levels of this function vary enormously within an individual over time, between individuals within the population and between populations. Adaptive responses of the human reproductive system to environmental and constitutional conditions can best describe this pattern of variation (Ellison, 1999). Many issues of reproductive epidemiology can be clarified by understanding these patterns.

2.5 Developmental Hypothesis

In a healthy, normal woman, ovarian function matures gradually after menarche until the mid-twenties and remains relatively static thereafter until the late thirties; it then declines gradually until the menopause. In a study of healthy women, Lenton et al., (1984) found a progressive shortening of follicular phase length which was longest among the women in their early twenties and shortest in their forties. This three-phase trajectory of female ovarian function (postmenarche, prime reproductive life, perimenopause) is familiar to every population despite varying ecological, geographic and genetic backgrounds. For example, patterns of variation of age-specific ovarian function (salivary progesterone) among Boston women (Lipson and Ellison, 1992), Lese women of Zaire (Bailey et al., 1989; Ellison et al., 1989) and

Tamang Nepali women (Panter-Brick, 1993) displayed similar parabolic trajectories with age. However, there is variability of levels of function and age ranges across populations such as Lese and Tamang women who had lower levels of age-specific progesterone compared to Boston women (Ellison, 1994).

Figure 2.1 Age variation in mid-luteal salivary progesterone in three populations with best-fit quadratic regression lines. (Ellison et al., 1993)



(A) Boston (n = 136); (B) Lese of Zaire (n = 144); (C) Tamang of Nepal *(n* = 45); (D) comparison of the three regression lines with data points removed.

In order to explain variability in reproductive function and age at maturity, Ellison (1996) suggested: *"levels of ovarian hormonal function in adult women are associated with the tempo of growth and maturation in childhood and adolescence"*. In other words, developmental conditions in early life influence later reproductive function. Therefore, energy availability during the growth and developmental stages of women determines adult levels of reproductive function, and variation in this energy availability in early life results in variation of later reproductive function. There are a few studies that have supported this hypothesis. For example, Apter and Vihko (1983) found higher reproductive hormonal levels in later life in women who had an earlier age at menarche than those with late menarche, while the incidence of ovulatory failure (Venturoli, 1987), oligomenorrhoea and dysmenorrhoea (Gardner, 1983) was higher in late maturing women. Therefore, it is evident that there is an intimate relationship between developmental tempo and adult ovarian function.

Relationships between early developmental conditions and both structural and physiological characteristics of reproductive function have been recognised. Among a Spanish cohort, it is evident that smaller and low birth weight female babies have smaller ovarian and uterine sizes (Ibanez et al., 2003) and have

lower reproductive hormonal levels during their later life (Ibanez et al., 2000, 2002). Similar observations by Jasienska et al. (2006) suggested that oestrogen levels during adult life are related to the ponderal index of Polish women at birth. The impact of adversity in early developmental life (such as nutritional deprivation) on future reproductive effort and later reproductive success was also evidenced by Lumey (1992) who found that low birth weight babies were born to mothers during the Dutch famine (1944-1945). Similarly, Cresswell et al. (1997) found an earlier menopause in women born with low birth weights and low weight gain in early childhood. Hardy and Kuh's studies (2002, 2005) also support the findings that early childhood weight gain results in a later age at menopause.However, relatively little is known about how female reproductive life may be affected by developmental conditions during childhood and particularly the slow growth period, though it is well evidenced that early life effects on growth are likely to have an impact on later reproductive capacity.

Although in the developmental hypothesis Ellison (1996) suggested that environmental conditions, the growth spurt and tempo of maturation during the developmental period influence later life ovarian function, there are alternative concepts that can be contrasted. These suggest some background influence like genetics. Therefore, examining a genetically similar population

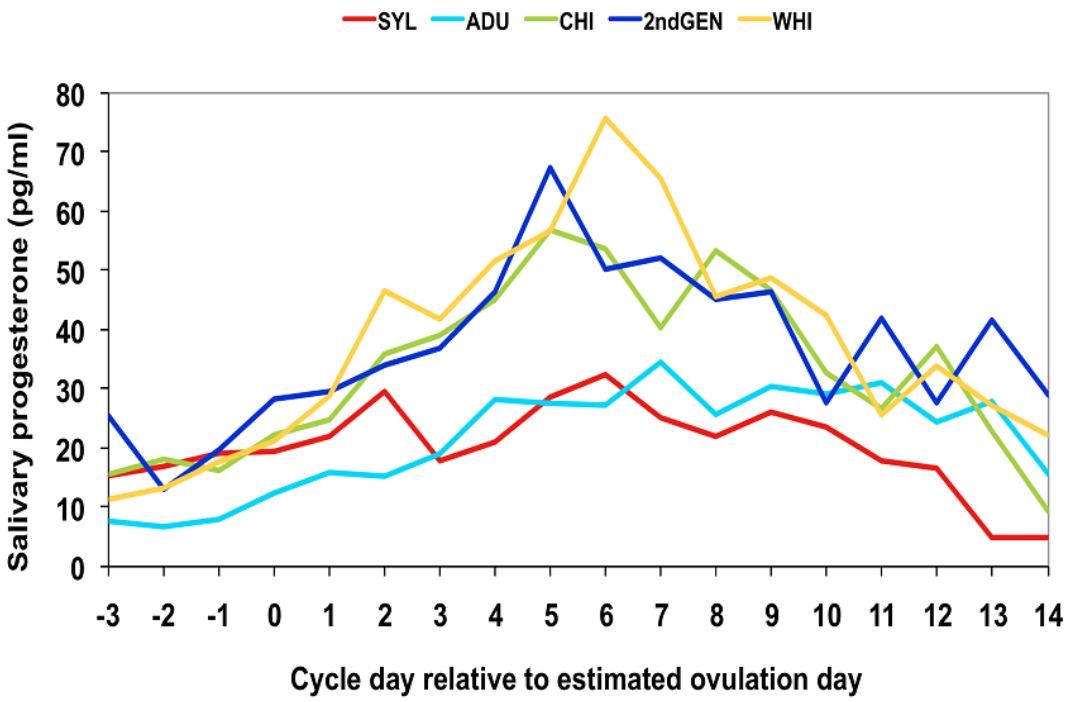
that developed and lives in a contrasting environment and comparing it to a genetically diverse population in the same environment may give an opportunity to identify crucial points of developmental stages when a change of environment has a significant impact.

Therefore, migration studies are the ideal model to examine the association between developmental tempo and adult reproductive function while controlling for genetic factors. But few studies have been carried out in this regard. Noticeable variation in reproductive steroid levels has been found in studies among women of reproductive age living in stressful conditions including poor nutritional intake, high energetic outputs and heavy disease loads.

For example, one study of middle class young Bangladeshi migrant women aged 19-39 years in the UK, Nunez de la Mora et al. (2007) found that luteal phase progesterone levels of adult Bangladeshi migrants were similar to their sedentee counterparts in Sylhet, Bangladesh (Figure 2.1), but were lower than Bangladeshi women who migrated as children as well as second generation Bangladeshis in the UK. The progesterone levels of the latter two groups were comparable to white British women (Nunez et al., 2007). They also found that progesterone levels were higher among women who had an earlier age at menarche across

the groups, while age at menarche was significantly correlated to age at migration among the child migrants. Furthermore, they found that salivary progesterone levels of the child migrants were positively correlated with the length of time spent in the UK prior to puberty.

Figure 2.2 Salivary progesterone profiles by the study groups over one menstrual cycle (Nunez et al.2007)



SYL=sedentee, ADU= adult migrant, CHI = child migrant, 2ndGEN = second generation Bangladeshi and WHI = European

Lower progesterone levels of the women who grew up in Bangladesh seem to result from poorer rates of growth and maturation in early life. These may be due to the adverse environmental conditions like higher disease loads and chronic immune challenges due to the persistent exposure to parasitic and microbial infections that prevail in Bangladesh (Nunez et al.,

2007; Nunez et al, 2008, BBS, 2005). However, the studied population was well fed with very low levels of energy expenditure (Nunez et al., 2007; Nunez et al, 2008). Therefore, the findings of low progesterone levels matching sedentees, and lower hormone levels compared to child migrants, second generation Bangladeshi migrants, and white British women suggest that adult migrants carry a slower developmental trajectory from their country of origin. The study was restricted to young migrant Bangladeshi women aged 18-35, and we do not know whether there are any effects in later reproductive life.

2.6 Mechanisms that might explain developmental effects

Although the hypothalamic-pituitary-gonadal (HPG) axis controls ovarian function, a negative feedback control regulates this axis. Therefore, hormone levels in adult life suggest an adjustment at different levels of regulation of the HPG axis. Differences in hormonal production might occur due to variation in levels of HPG stimulation or a variable sensitivity of HPG to negative feedback control. Adjustment of the feedback mechanisms could occur through two pathways: 1) a gradual decline in HPG sensitivity to negative feedback control (the Hypothalamic Desensitisation Hypothesis); and 2) gradual enhancement in gonadotropin drive to steroid production (the Direct Drive Hypothesis) (Ellison, 1996). As menarche is a stage

of the developmental process when reproductive steroid hormones reach a critical level to produce endometrial proliferation, variation in menarcheal age can be explained by either of these mechanisms. For example, individuals whose HPG axis is either constantly more sensitive to negative feedback or who have a lower gonadotropin drive achieve menarche at a later age. Therefore, constant variation in sensitivity to feedback or to the gonadotropin drive at an individual level suggests an association between developmental tempo and adult hormonal levels.

From a life history perspective, the metabolic axis plays a crucial role in the energy allocation of the growth, maintenance and reproduction, which may establish developmental trajectories. Environmental conditions in terms of energy availability, immunological challenge and physical factors during crucial periods of development determine the set points for metabolic activity that regulates developmental trajectories and thereby influences maturational tempo subsequent to adult reproductive function.

Metabolic hormones such as insulin, insulin like growth factor 1 (IGF1) and leptin play important roles in human growth and development. Insulin and IGF1 regulate growth and promote maturation through stimulating the HPG axis during the developmental period, and also have anabolic effects in adults

(Poretsky et al., 1985; Samoto et al., 1993; Karlsson et al., 1997; Duleba et al., 1998; Poretsky et al., 1999). Moreover, these hormones stimulate steroid production by the granulosa and theca cells in the ovary (McGee et al., 1996), and also stimulate oocyte maturation and follicular growth (Willis et al., 1996). Leptin, a hormone released by adipose tissue, also regulates GnRH and LHRH secretion through stimulating the HPG axis (Mcmillen and Robinson, 2005; Gluckman et al., 2007). Several studies suggest that these hormones are crucial for establishing set points for pubertal development, the tempo of the adrenarcheal and pubertal developmental spurt, and the simultaneous rise of adrenal activity (Apter, 1997; Wilson, 1998; Foster & Nagatani, 1999; Gluckman et al., 2007). Leptin helps to maintain the normal menstrual cycle and reproductive function. Levels of IGF1 and leptin significantly increase before puberty (Poretsky et al., 1999).

2.7 Proposed study

From the above findings from studies of younger women, it is evident that reproductive function in later life is dependent on developmental trajectories in early life. Female reproductive function declines with age and depends on an ovarian reserve that is established in early life. *"All follicles in the ovary, from primordial to preovulatory, are considered part of the total pool, termed ovarian reserve"* (Neal-Perry and Santoro, 2006). Ovarian

reserve can be predicted by measuring three reproductive hormones: follicle stimulating hormone (FSH), inhibin B and anti-müllerian hormone (AMH). The normal pattern of these hormones with increasing age are increasing FSH and decreasing inhibin B and AMH. As mentioned above, ovarian function and reproductive hormones vary between populations depending on early developmental conditions. Accordingly, age-related ovarian reserve should also be variable between populations. Therefore, women who develop in adverse environments could be expected to have lower age-related ovarian hormones (inhibin B and AMH) and higher levels of age-related FSH than those who developed in better conditions, but migration to better conditions after maturity (after menarche) should not affect age-related hormonal levels between migrant and native populations. Although variation in hormonal levels has been found in different ethnic populations, a study comparing a migrant population with the host population and population of origin might provide a better understanding of variability in ovarian reserve between migrant and host populations and might give a more clear picture of developmental effects on reproductive biology, as well as any effect of environmental change.

In accordance with the findings of the previous study on young Bangladeshi women (Nunez de la Mora, 2007), the present

study was developed to examine Ellison's "Developmental Hypothesis" in the context of Bangladeshis who migrated to the UK during adult life, Bangladeshis who migrated to the UK as children, sedentee Bangladeshis still living in Bangladesh, and white women of European descent aged between 35 and 59 years. The study was carried out with the assumptions that adult migrants and sedentees would have a lower age-related ovarian reserve compared to child migrants and women of European descent since women who developed in Bangladesh experienced an environment that is more physiologically stressful with higher disease loads, poor hygiene and immunological challenges.

CHAPTER 3

STUDY POPULATION

This chapter deals with background information in relation to selecting the Bangladeshi population for the study. The first section of this chapter gives an overview of background information and history of migration from Bangladesh to the UK. The second section presents the socio-demographic characteristics of the study population. Finally the possible clues about the contrasting environments between Bangladesh and the UK, and the rationale for selection of the study population are explained in the last section.

3.1 Background and history of migration

Bangladesh is a small South Asian country with an area of 147,570 square kilometres and a population of about 130 million (BBS, 2005), and is one of the most densely populated countries at about 1,026 people per km². It is ethnically homogeneous, with 98% Bengalis. Administratively, Bangladesh is made up of six divisions which are divided into 64 districts and then 507 smaller units called sub-districts (*thana* or *upazilla*) which are further divided into unions, and within each union there are villages of different sizes. The district is the administrative unit while the *thana* is the local government unit.

The economy of Bangladesh is based on agriculture and human settlement is primarily rural based. Although most of the people live in the rural areas (79%), frequent internal migration to urban areas occurs. Despite domestic and international efforts, Bangladesh remains an underdeveloped and overpopulated country (World Bank, 2005). However, in spite of the socio-political hurdles, there has been a slow but steady increase in gross domestic product (GDP) per capita with an average annual growth rate of 5% since 1990 (World Bank, 2005), although about 50% of the population is still poor with no urban-rural variation. Thirty six percent of the population is living with a per capita income of below US $1 a day. Due to a strong family planning programme, the annual population growth rate declined from 2.33% in 1981 to 1.50% in 2002 (BBS, 2002). Similar declining trends are seen over the period of 1981-2002 for the crude birth rate (34.4% vs 20.9%), crude death rate (11.5% vs 5.9%) and total fertility rate (5.24% vs 3.0%) (BBS, 2002). In Bangladesh, 38% of the population is under 15 years, 55% is in the age group of 15-59 years, and 7% is in the age group of 60 years and above (BDHS 2004).

The average life expectancy in Bangladesh has improved from 55 years in 1981 to 65 years in 2002, with 64 years and 65 years for males and females respectively (BBS 2002). A

considerable decline has occurred in the Infant Mortality Rate (IMR) during 1981-2002. In 1981, the IMR was 111 infants per 1,000 live births, which reduced to 87 in 1991, and 53 in 2003 (BBS, 2003). In Bangladesh, the maternal mortality rate has declined from 4.7 women per 1000 live birth to 3.9 per 1,000 live births in 2002 (BBS, 2002). About 69% of the population suffers from iodine deficiency disorders and the prevalence of anaemia among adult women was estimated at 74 %. The prevalence of low birth weight (weight <2500 grams) has decreased from about 50% in 1995 to 40% in 2005. The adult literacy rate in the population over 15 years of age has shown a gradual increase from 1981 (males 39.7% vs females 18.0%) to 2002 (55.5% vs 43.4% respectively) (BBS, 2002). However, Bangladesh remains one of the least developed countries in the world; 19.2% of its inhabitants live in extreme poverty with greater effects on women. In every respect, ranging from health and education to nutrition and income, women are the poorest of the poor.

Sylhet, the city where this study was focused in Bangladesh, is located in the northeast of the country beside the river Surma. Topographically it comprises mainly hills surrounding a few large depressions called *beel*. The climate is mainly hot, humid and wet tropical. As the city is situated in the monsoon zone, the average highest temperature is approximately 23°C (August-October) and

lowest approximately 7°C (January). Sylhet city covers 57.64 km^2 and has a population of approximately 0.4 million (BBS, 2007). The average literacy rate is 64% (68% male and 59% female) and the population growth rate is 1.75%; population density is about 1,136 per km^2.

The Sylheti population mostly uses the Sylheti dialect, which is derived from the Bengali language, but most people are bilingual with standard Bengali that is taught in schools and used for more formal occasions. Religiously, the population is mostly Muslim. There are some cultural differences between Sylhetis and other Bangladeshis. For example, they mostly marry within the Sylheti community. They are family-oriented and are more religiously conservative. They are mostly segregated due to a protective attitude to their language and strong family ties. Family ties also exist between their kin living in the UK. Moreover, most of the households in Sylhet have kin or a family member in the UK, while most of the migrant Bangladeshis (>90%) in the UK are from the Sylhet district. As large numbers of Sylhetis are living abroad, particularly in the UK and USA, major economic development is through remittances received from Sylheti families abroad, which have given rise to an affluent class in Sylhet town. Due to their unique cultural, economic development and difference in the language, Sylhet exists as a separate entity. Therefore, the

migrant Bangladeshi and Sylheti population are homogeneous with their socio-cultural and to some extent economic perspectives.

Migrant Bangladeshis are the fastest growing ethnic minority groups in the UK. In the UK, around half of the ethnic minority population are Asians, of which Indians are the largest of these groups, followed by Pakistanis, Bangladeshis and other Asians (ONS, 2002). Connected to the country's colonial past, they have a long history of migration to the West particularly to the UK. In the beginning, adult migrants who came to the UK were working in the British merchant navy and jumped ship when they found themselves near the coast (Alam 1998, Adams 1987, Carey and Shakur 1995). Subsequently, their experiences in the UK attracted Sylheti villagers and that encouraged middle-income families and small landholding families from Maulvi Bazaar, Biswanath and Beani Bazaar who could afford the travel expenses to come in the UK (Carey and Shakur 1985-86). Afterwards, more Bangladeshi adult migrants were able to come during the post- Second World War economic boom, when labourers were needed in every sector in the UK (Eade, Vamplew and Peach, 1996). During the 1960s, the new Commonwealth Law in 1962 created a voucher system for migration which was discontinued in 1964-65, but which effectively stopped all primary migration. In the 1970s, new

immigration rules restricted all entry except the closest relatives, and this began a process of consolidation of families in the UK. Therefore, the migration of the Bangladeshi population was previously related to a poor economic cycle. Although this trend is still continuing, the current motive for migration is largely for the formation or reunification of families.

According to national statistics in the UK (2005), there were 283,000 Bangladeshis living in the UK in April 2001, which is 0.5% of total population and 6.1% of the ethnic minority population (National Statistics, UK 2005). About 80% of the Bangladeshi population are living in urban areas, with about 75% living in three cities in the UK namely London, Birmingham and Manchester (Ali, 2000). However, about 54% of the total Bangladeshi population are living in London, which is about 3.8% its population. They are mostly concentrated in the boroughs of Tower Hamlets and Camden comprising 43% and 8% respectively of the borough's population (ONS, 2001,2002). In terms of age structure, a high percentage of Bangladeshis are in the 5-15 year old age group (31.9%). The average age of Bangladeshis as a whole is 17.1 years, and 64.9% of Bangladeshis are under the age of 25. Bangladeshis are now entering the third generation since migration (Eade and Momen, 1996).

3.2 Socio-demographic characteristics

The migrant Bangladeshi community is a rapidly growing ethnic minority population in the UK which has a demographically, culturally and economically distinctive character of a joint family, large household size and multigenerational composition. Migrant Bangladeshis are highly segregated from both the local community and other ethnic minority communities. Their high degree of segregation results from language barriers, a general low level of literacy of English, low level of academic and professional education causing lack of skill and unfamiliarity with the cosmopolitan living environment of London. Moreover, the strong male-dominated social structure prevents integration into the host community particularly for women due to a fear of exposure to western values and influence. All these factors have made them concentrate into particulars area like Tower Hamlets and Camden where they can keep their cultural identity.

3.2.1 Household size and number of children

National UK data suggest that the average family size of Bangladeshis in the UK is twice that of the white European population. Among ethnic minorities, Bangladeshis have the highest number of household members (4.7 persons/household), which is comparable to Bangladeshi statistics of 5 persons/

household (BBS 2002). Summerfield and Babb (2003) suggested that about 50% of the households of Bangladeshis in the UK have more than 5 people, while two-thirds of the white European population have less than 3 members in their house (DWP, 1999). On the other hand, 4 or more children in the family were found in about 50% of the Bangladeshi family in the UK, and only 4% among white European families (ONS, 2001).

The possible explanation related to the larger family size in the Bangladeshi population both residing in Bangladesh and in the UK is related to their distinctive culture of living in an extended family where the family comprises a husband's parents and siblings together in same household. In contrast, a considerable number (34%) of the white population in the UK are single. However, recent data suggest that cultural attitudes towards living with extended families in the migrant Bangladeshi population are changing and the possible explanation is the availability of smaller than desired accommodations.

3.2.2 Education

Migrant Bangladeshis in the UK usually have some elementary education obtained in Bangladesh. It is evidenced that, in general, Bangladeshis particularly women have a low level of education and literacy in both language and professional

qualifications. The scholastic aptitude of migrant Bangladeshis was found to be lowest among all ethnic groups (Brooker, 2003; Ghuman, 2002; ONS, 2002). Bangladeshis in the UK are most likely to be unqualified, with 40% of males and 48% of females having no qualification at all. About 37% of girls and 22% of boys get qualifications up to GCSE (grade A*- C), which is the lowest proportion among ethnic populations in the UK (National Statistics, UK 2005).

The possible explanation for this discrepancy is due to migration of a larger proportion of women from a rural background where educational opportunities are limited. Moreover, migrant women are mostly married at a younger age than their UK resident kin and the marriages are mostly arranged at the family level.

3.2.3 Economic condition

Adult migrants experience a major change in their socio-economic status from being a landowning, middle-income group in a poor country to an economically deprived status in a prosperous, post-industrialised and urban environment. About 22% of males and 24% of females of the total Bangladeshi population are reported to be unemployed, which is 4 times higher than the white European population, while 40% of young (under

25) Bangladeshis are unemployed (National Statistics, UK 2005). The women are considered economically inactive as they are mostly involved in household work including looking after the family, as they do not generate income. By occupation, more than 60% of employed Bangladeshis are working in the hotel and restaurant industry. Among ethnic minorities, the Bangladeshi population has the highest economic inactivity (not available for work or actively seeking work) and two thirds of the inactivity is related to long term sickness and/or disability (National Statistics, UK, 2005).

3.2.4 Accommodation and Housing

According to national statistics, UK 2005, Bangladeshis households are least (37%) likely to own their home and 48% lived in social housing which is the highest figure for this dependency of any ethnic group in the UK. In contrast, the white European population is less dependent on social housing (20%) while 70% own their house. However, the accommodation pattern in Sylhet is quite different from the UK. Until recently, the accommodation pattern in Sylhet was large houses resembling cottages. However, recent economic changes from remittances and growing property development have brought in the more western concept of apartments.

3.3 Selection of study populations

Despite having some differences in socio-economic status, Bangladeshis in the UK have several characteristics that make them an exceptional study population. As previously mentioned, more than 90% of adult migrants come from Sylhet district (Gardner, 1995), which is ethnically homogeneous and geographically concentrated. The possible explanation for the homogeneity and geographically concentrated migration is caused by the fellow feeling due to Sylheti culture and dialect that prompted the chain migration process of Bangladeshis to the UK. As the purpose of migration in recent days is mostly for reunification of families and marriage with other ethnic groups rarely occurs, there is reduced possibility of genetic confounding that can be an important aspect of a migration study. Sylhetis also constitute a multi-generational population who are now continuing to the third generation (Eade et al., 1996). The Sylheti population who migrated to the UK are from the small, landowning middle class (Gardner, 1995) and they continue a similar life style to Sylhet after migration. For example, the dietary habits of adult migrants are similar to their native counterparts in that it is mainly comprised of rice and Bangladeshi curry, although adult migrants usually take fewer vegetables compared to sedentees. Therefore, their diet is composed mainly of high carbohydrates

and fat.

Environmentally, Sylhet differs from the UK in the disease load experienced by residents, pollution, hot and humid weather, inadequate sanitation, poor water supply, and immunological challenges. For example, in Sylhet, only 25% of the population have access to water supplied by the municipal corporation, while the rest (75%) either use tube wells or pond water. Furthermore, only 6% of people have access to a proper sanitary latrine, 27.5% use a low cost sanitary latrine, while the rest of the population do not have access to a sanitary latrine (unsanitary latrine). Overall the drainage facilities of the Sylhet town are very poor and there is no solid waste disposal facility. Therefore, latrines become the source of faecal contamination during floods (Ahmed et al., 2010). Although the population of this study were drawn from the middle class who does have access to clean water and sanitary latrines, the town of Sylhet is subject to seasonal flooding during monsoons that make an unsanitary environment where infection and disease are endemic.The widespread infectious disease and parasitic infestation causes both symptomatic and asymptomatic disease morbidity.

On the other hand, the overall environmental situation in the UK is far better with a clean water supply, access to good health services, adequate sanitation and less pollution. Despite

having such differences between the two communities (Bangladeshi in London and in Sylhet), Bangladeshi adult migrants are mostly living in a concentrated geographical boundary in London (Tower Hamlets and Camden) and still maintain their social, cultural and traditional values. The pattern of family formation is homogenous within the Bangladeshi community. There are almost no Bangladeshi women married to non-Bangladeshi men (Diamond & Clarke, 1989; Eade et al., 1996a), so there is no chance of genetic confounding. Therefore, Bangladeshi migrants comprise an ideal study population for this hormonal study.

Ecologically, therefore, Bangladeshi adult migrants move from a developing country with poor sanitation, high rates of infectious diseases and water borne parasitic infection to a developed country. Infectious diseases are the major cause of morbidity and mortality in Bangladesh, which result from poverty, population density, poor sanitation, malnutrition, and disease-transmitting insect vectors. Diseases with infectious aetiologies include pneumonia, diarrhoeal diseases, tuberculosis, measles, vector-borne diseases like dengue, malaria, visceral leishmaniasis (*kala azar*) and filariasis. Intestinal parasitic infection prevalence is quite ubiquitous in Bangladesh. Different surveys conducted on rural samples, hospital patients, students, urban slum dwellers

and tea garden workers suggest a prevalence of round worm ranging between 70% and 95%, whipworm between 38% and 79%, and hookworm between 2% to 71% (Mutalib et al., 1976; Hall et al., 1992).

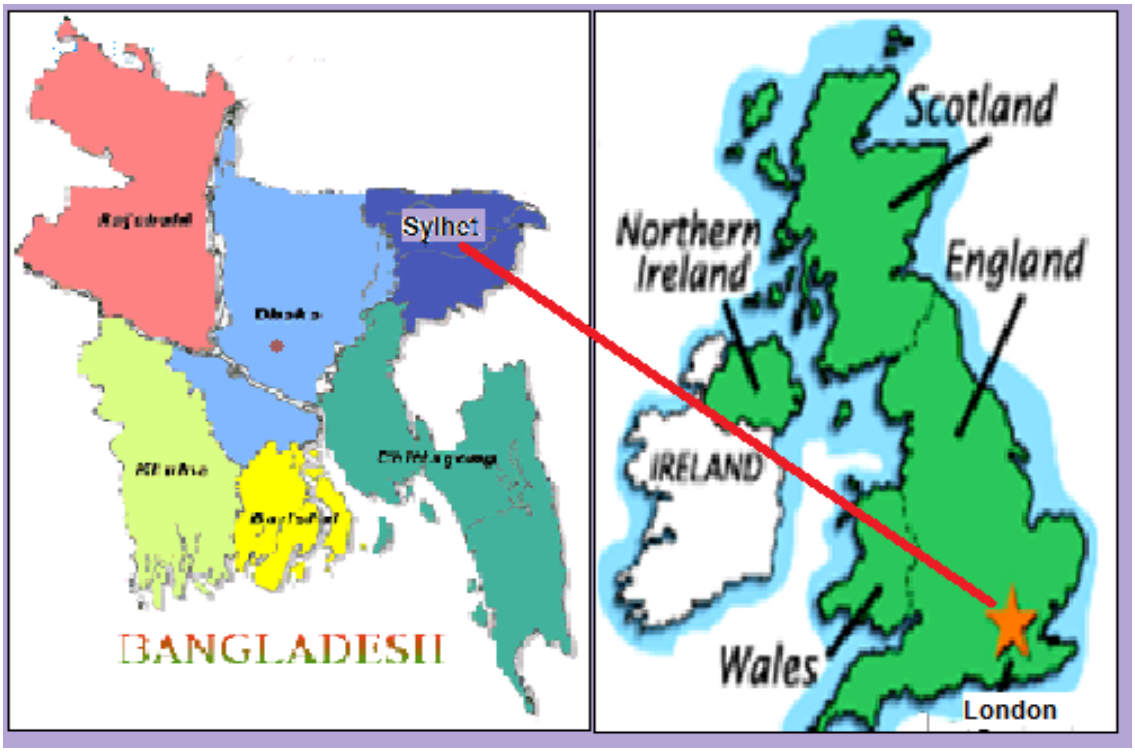
Infection with multiple parasites (polyparasitism) is quite common in Bangladesh and co-infection occurs with roundworm, whipworm and hookworm (Hall and Nahar, 1994, Rousham and Mascie-Taylor, 2001, Mascie-Taylor and Alam 1997, Giligan and Masie-Taylor, 2001). For example, of women tea pluckers working in the Sylhet tea gardens only 10% were free from worms, 27.5% had single infections, 33% had double infections and 26.6% were infected with all three worms (Giligan and Mascie-Taylor, 2001). The possible causes were identified as poor sanitation, unclean water and lack of personal hygiene (Mascie-Taylor, 1996).

In contrast, the UK Bangladeshis are exposed to a better environment with clean water supply, hygienic sanitation with proper disposal system, less pollution, less humid weather, and therefore, they have a lower immunological challenge. Moreover, having migrated from a confined geographic area, they are still living in a concentrated geographic area in London with a similar cultural and social environment to Bangladesh. Therefore, the findings of this study are not only of anthropological interest but

may also shed lights on issues of public health, specifically those concerned with ethnic minorities.

Figure 32.1 Map of Bangladesh

Figure 3.2 Migration from Sylhet to London



CHAPTER 4

METHODS AND MATERIALS

This study is part of a bigger ongoing National Science Foundation (NSF) funded collaborative, bio-cultural study on menopause and symptom experience. The collaboration is between University College London (UK), Durham University (UK), the University of Massachusetts, Amherst (USA) and Sylhet MAG Osmani Medical College (Bangladesh). This study has focused on Bangladeshi migrants in London, sedentee Bangladeshis living in their community of origin, and women of European descent in London. It was carried out between September 2006 and August 2010.

4.1. Subjects

4.1.1. Composition of groups

The study has been carried out on three different groups of populations:

Group I: Sedentee Bangladeshi women living in Bangladesh who were born and brought up there (SYL) (n=45).

Group II: Women born and brought up in Bangladesh who migrated to London as adults aged >16 (ADU) (n=57).

Group III: Bangladeshi Women who migrated to London as children aged ≤16 (CHI) (n=53).

Group IV: Women of European descent living in London (EUR) (n=52).

The volunteers were healthy women who were premenopausal, perimenopausal or postmenopausal. According to WHO, premenopause is defined as where menstruation has occurred within the past 2 months, perimenopause as where menstruation has occurred between the past 3-12 months with irregularity in cycling, and postmenopause as where permanent cessation of menstruation has occurred with at least 12 consecutive months of amenorrhoea (WHO, 1981).

To include all three menopausal stages, women were recruited between the ages of 35 and 59 years. To ensure an equal age distribution of the sample population, women were divided into 5 age categories: 35 to 39, 40 to 44, 45 to 49, 50 to 54 and 55 to 59 years, with an ideal minimum of 8 women from each age category recruited across the groups. However, for the child migrant (CHI) group, there are no women in the age category 50-54 and 55-59 years due to the specifics of Bangladeshi migration history (see Chapter 3). A total of 207 women were finally recruited across all groups with 45 sedentees

(SYL), 57 adult migrant Bangladeshis (ADU), 53 child migrants (CHI) and 52 Europeans (EUR). Although more than 40 samples were collected from each study group, the sample could not be collected in equal number across the age categories as expected.

4.1.2 Recruitment

To recruit women from different groups, several strategies were used including individual contact through friends or relative, public contact through community centres, and advertisements in newspapers or websites, as well as flyers and leaflets. The strategies adopted for each group are detailed below.

Bangladeshi migrant women: Women from this group were recruited from the Bangladeshi community in North London, including Euston, Camden Town, King's Cross and Islington areas, and from the East London neighbourhoods of Whitechapel, Stepney Green, Mile End, Bethnal Green, and Upton Park. Women were recruited through different connections such as Bangladeshi community centres, associations, melas (gatherings or fairs, as used in the Indian subcontinent which can be religious or cultural), social gatherings like an Eid (a religious holiday) reunions, cultural programmes and health clubs. Several government-funded Bangladeshi community centres in these London areas are used

by Bangladeshi migrants for social, health and education purposes.

Liaisons with these links were maintained through conducting health sessions and providing health-related advice and information to the women who usually came to these institutions. A stall was introduced at the melas and other different festivals held in the Bangladeshi community. Health-related advice and nutrition-related information were given to women who attended these occasions. Blood pressure, height, weight and BMI were measured as a part of health advice. After building rapport with the women who came to these community centres and festivals, women were introduced to the project and interested volunteers were recruited.

Women were also recruited through leaflets and flyers that were distributed in the community centres, melas and local shops and pharmacies. Moreover, recruitment was also carried out using a snowball technique that included personal referrals from previously recruited women and word of mouth. Furthermore, recruitment through advertisements in the freely distributed London newspapers (Metro) was done. Communication with the women was maintained by telephone. A Bengali information sheet was distributed to those who were interested in gaining a better

understanding about the project. Specific questions and concerns were clarified accordingly by the researchers.

The women were mostly screened by administering a screening questionnaire over the telephone (Appendix I). Prior to data collection, eligible women were asked to read an information sheet and sign a consent form. The interview and blood draw (on the 4^{th}, 5^{th} and 6^{th} day of the cycle from the women who were menstruating and at any time from the women who were not menstruating) were usually performed at a place where it was convenient for both women and researcher such as in a community centre, a woman's house, or at the Anthropology Laboratory, UCL.

Women of European descent: Women of European origin (EUR) who grew up in the UK and live in London were recruited through advertisements in local free newspapers (Camden Journal and Metro). Information about the research and a call to participate in the study were also posted on the website "Foreignlondon.com".

Screening questionnaires were administrated to the interested woman over the telephone or through e-mail. Women who were eligible were then recruited for the study and were scheduled for the interview and blood draw. Women were asked to

read and understand the information provided in the information sheet and to sign a consent form. The women were interviewed by administrating main questionnaires. Five ml blood samples were collected on the 4th, 5th or 6th day of the cycle from the women who were menstruating and any time from the women who were not menstruating. The interview was usually held at the Anthropology Laboratory, UCL and the blood draw was performed in the laboratory at the Centre for Reproductive Science, UCL.

Sedentee Bangladeshi women in Sylhet: The sedentee Bangladeshi samples were collected from Sylhet town, Sylhet, Bangladesh, as migrant Bangladeshis to the UK originate mostly from here. Only Bangladeshi women with the means to emigrate were included in the study for comparison with immigrants in London. The fieldwork was carried out between March and April 2007 in collaboration with Professor Osul Chowdhury at Sylhet MAG Osmani Medical College. A team of students from Shahjalal University, Sylhet, assisted in screening women who were interested in participating in the project. In some areas, women were introduced by local volunteers who were popularly known and had a good rapport in those areas. These local volunteers were either bankers, schoolteachers, or other prominent members of the community.

The protocols that were used to screen and interview women in SYlhet were identical to those described above (page 83-84) except that the main interview was held at the Sylhet MAG Medical College, the subject's house or a school according to the subject's and the researcher's convenience. Blood was drawn in the Microbiology Laboratory, Sylhet Medical College.

4.1.3 Screening of the subjects and eligibility criteria

The screening and recruitment of women were done by several researchers, including myself, through a team effort. A short, structured screening questionnaire was administrated to each healthy woman across the four groups to assess the eligibility of women to participate in the study (Appendix I). Standardised inclusion and exclusion criteria were set for participation in this study. The criteria were as follows:

- Participating women must be aged between 35 and 59 years. This age range was selected to include women at the initiation of an FSH rise -- in other words at the beginning of the menopausal transition -- which usually occurs in the early 40s, but sometimes in the late 30s, (Santoro, 1996) and to also include women with a late menopause.

- Women must not have a history of hysterectomy and/or oophorectomy. Exclusion of these women would give a better understanding of the natural menopausal transition.
- Women must not be pregnant within the past six months.
- Women must not be lactating within the past six months.
- Women must not be taking exogenous hormones such as hormonally-based contraceptives (oral, implant or IUD-based) or hormone therapy for any other cause within the last six months. Hormone therapy is very likely to alter the hormonal data and diminish the experience of menopausal symptoms such as hot flushes.
- Women must not suffer from endocrine diseases such as diabetes or thyroid problems as these might interfere with hormone levels.
- Women with polycystic ovarian syndrome (PCOS) were excluded from this study as this disease interferes with normal ovarian hormonal levels.
- Both parents of the woman must come from same ethnic group to exclude genetic confounders.

4.2 Data collection

4.2.1 Questionnaires

All the recruited women were interviewed extensively through administering a standardised, structured questionnaire with both open-ended and closed-ended questions (Appendix II and III). The questionnaire was divided into two parts. The first part contained questions concerning personal details, socio-demographics, education, employment history, migration history (applicable only to migrant Bangladeshis), and menopausal history. The second part of the questionnaire was related to general health and reproductive histories.

For Bangladeshi women who did not know their actual age, an event calendar using important national memorable occasions, such as the War of Independence, Victory Day, the India-Pakistan War in 1965, major natural disasters (e.g., a cyclone in 1970) was devised to calculate actual ages for women. To determine age at menopause, women were asked to remember the season of the year or any family, political or national event in relation to their last menstrual period. Socio-demographic information on the questionnaire included educational status, financial conditions and accommodation. For educational information, women were asked about number of years of school attended, and where they were

educated. This varies particularly for migrant Bangladeshis - mostly they were schooled only in Bangladesh but some were schooled both in Bangladesh and the UK.

To assess financial condition, the women were asked about their perception of their household financial condition, which was categorised as "struggling", "OK", "comfortable" and "well off". They were also asked about whether they owned a house or car. Information about accommodation comprised type of house and ownership of the house. Migration history included questions related to age at migration and year of migration which was used for computing years in the UK.

Reproductive history included a woman's menstrual history and obstetric history. For menstrual history, the women were asked about whether they were menstruating or not. For women who were menstruating, they were asked about their menstrual regularity, length of menstrual cycle, age at menarche. For menopausal women, they were asked about age at menopause and date of their last menstrual period (LMP). The information was used for computing the subject's current menopausal status. Menopausal status was calculated by computation of date of interview minus the month or year of the last menstrual period (LMP) and was categorised according to WHO definition (see

section 4.1.1, page 80). Obstetrical history comprised questions about number of pregnancies, and number of children.

4.2.2 Anthropometric measurements

Height and weight measurements were taken from each subject to compute BMI. All these measurements were taken thrice and the average was recorded as a final measurement. Height was measured in centimetres (cm), while weight was taken in kilograms (kg). Height measurements were taken to the nearest tenth of a cm (0.1cm) or 1.0 millimetre (according to manufacturer's guideline), while weight measurements were taken to the nearest tenth of a kg (0.1kg) (according to manufacturer's guideline) (Lohmann et al., 1988)

Height measurement: Height was measured using a stadiometer. A standard procedure was followed according to manufacturer's instructions. The women were asked to remove their shoes and stand on the footboard or floor in the erect position with their back against the vertical stand, heels placed together and feet placed outward at a 60º angle. The heel, buttocks and back of the head were pressed against the vertical stand with hands hanging freely by the side of the body. Measures were taken with participants standing in a fully erect posture. The

horizontal head board or scale was pressed gently on the top of the head so that the hair compressed sufficiently.

Weight measurement: A portable weighing scale was used to measure weight. Women were asked to stand on the centre of the scale after removing shoes and all heavy clothing. BMI was computed using the following formula:

BMI = weight in kg/ (height in meters)^2.

Anthropometric indices of BMI were categorised according to WHO where BMI <18.5 =underweight; 18.5-24.9 = normal; 25-29.9 = overweight; >30 = obese.

4.2.3 Blood sample collection

Blood samples were collected from all women who were menstruating on cycle day 4-6, and at any time from non-menstruating women. Cycle day 4-6 was chosen for blood collection from the menstruating women as discussed in Chapter 1 (Section 1.4, Pages 20), because serum inhibin B and basal FSH levels peak during this period (Muttukrishna, 2004). Therefore, serum levels of inhibin B, FSH and AMH during this period can predict the ovarian reserve (Durlinger, 2002a). Prior to the blood sample collection from each woman, the beginning of the menstrual period for that cycle was confirmed from the women verbally and then the day of the blood collection was calculated.

During recruitment I documented women's menstrual histories i.e. last menstrual period, cycle regularity and cycle length. For the blood sample collection, I contacted the women a couple of days before the possible beginning of their next menstrual cycle after calculating this from their menstrual history, and kept contacting them everyday onwards until the start of their next cycle. In some cases, women contacted me when they started their menstruation. An appointment was then scheduled for blood sample collection on either of day 4, 5 or 6 according to the woman's convenience. Before blood sample collection, women were asked again about their menstrual history and this was cross checked with the previously reported history to check for errors.

In London, blood samples were collected in the laboratory at the Centre for Reproductive Science, UCL, where the samples were centrifuged and pipetted. Separated serum samples were kept at -20º C in the same laboratory until analysis. In Bangladesh, blood samples were collected in the microbiology laboratory at Sylhet Medical College, where samples were centrifuged and pipetted. After separating serum from the collected blood samples, the serum samples were kept at -20º C in the same laboratory until transported on dry ice to UCL, London.

Blood collection and serum separation: Five ml of blood was collected by venipuncture from the antecubital vein using a vacutainer (BD Vacutainer Z REF 366636) with a 21 G needle. A tourniquet was placed in the middle of the arm and tightened to the limit a subject can tolerate. The vein was felt in the ante-cubital fossa at the front of the elbow joint. The area was swabbed with isopropyl alcohol prior to needle insertion. After confirming the needle was in the vein, the vacutainer tube was introduced at the other end of the needle. When the tube was filled with the desired amount of blood, the tube was withdrawn. Then the needle was withdrawn and the puncture site pressed with cotton and covered with a plaster. The procedure maintained aseptic precaution.

After collecting the blood, the vacutainer tube was kept standing for at least 10 minutes. The sample was then centrifuged at 3,000 rpm for 10 minutes. The serum was then separated using a pipette and put in Eppendorf tubes. Separated serum samples were then kept at -20ºC in laboratory at the Centre of Reproductive Science, Department of Obstetrics and Gynaecology, UCL, prior to hormonal analysis. Blood samples collected in Bangladesh were separated in the Microbiology Laboratory of MAG Osmani Medical College, Sylhet, and stored in the same laboratory until transported on dry ice to UCL. Hormonal analyses were done

in batches at the UCL laboratory after collecting a sufficient number of blood samples. All blood samples from Bangladesh and London were collected and centrifuged by myself.

4.2.4 Hormone analysis

Blood samples were analysed for FSH, inhibin B and AMH.

Follicle Stimulating Hormone: Serum FSH levels were analysed using an electrochemiluminescence immunoassay kit manufactured by Roche Molecular, Biochemicals, Mannheim, Germany. Mean intra and inter-assay CV<10%. Measuring range of the assay is between 0.10 mIU/L and 200.00mIU/mL (See Appendix 2 for details of the procedure).

Inhibin B: This hormone analysis was performed using an enzyme-linked immunosorbent assay (ELISA) kit for the quantitative measurement of dimeric inhibin B in human serum, manufactured by Diagnostic System Laboratories, Texas, USA (DSL-10-84100). Minimum detection for human recombinant inhibin B was 10pg/ml. Mean intra- and inter-assay CVs were 6.2 and 7.2% respectively.

Anti-müllerian hormone (AMH): Serum AMH levels were analysed using an ELISA kit manufactured by Immunotech SAS, Marseille, France (A 16507). Sensitivity was 0.42 pmol/l. Intra-

and inter-assay variations CV were <15% using in-house quality control pools.

4.3 Ethical permission, informed consent and data protection:

Ethical permission was obtained from University College London Ethics Committee, UK, the Ethics Committee for the Department of Anthropology, Durham University, UK, the University of Massachusetts, Amherst Internal Review Board (IRB), USA, and the Ethics Board, Sylhet MAG Osmani Medical College, Bangladesh.

An information and consent sheet was developed in both English and Bengali and was distributed to interested participants. Translation to Bengali was done by an independent professional who had a good understanding of both Bengali and English, and a back translation was performed by another independent professional.

Collected data were stored and managed according to the Data Protection Act (UK).

4.4 Data Analyses

4.4.1 Power and sample size issues

The main project included six groups: 1) Sylheti women who migrated to London as infants (0-2 years); 2) Syheti women who migrated as children (3-8 years); 3) Sylheti women who migrated as pre-adolescents (9-menarche); 4) Sylheti women who migrated to London as adults (post-menarche); 5) Sylheti sedentees still living in Bangladesh; and 6) white women living in the same London neighborhoods as the Bangladeshis and born in the UK to white British parents. An a priori power analysis for ANOVA was carried out to estimate required sample sizes using G*Power software (Erdfelder E, Faul F and Buchner A, HEINRICH HEINE University, Dusseldorf, Germany). With alpha set to 0.05, power set to 0.80, 6 groups and a conventional medium effect size (Cohen's f = 0.28, adjusted from 0.25), a required sample size of 240 (40 per group) was calculated. As this proposed study deals with only four groups (SYL, ADU, CHI and EUR), the sample size was 160 (40 per group). To A total of 207 blood samples from four groups were obtained, numbering 45 from SYL, 57 from ADU, 53 from CHI and 52 from EUR.

4.4.2 Statistical analyses

The data were checked, cleaned and verified against the original questionnaires. Continuous variables were checked for distribution, linearity and presence of outliers. Continuous variables such as age, BMI and education (year of schooling) were either used as such or transformed into categorical variables for some analyses. Age was categorised into five groups, which were 35 -39, 40-44, 45-49, 50-54 and 55-59 years. The age categories were designed to be homogeneous in distribution across the study groups although this was not always possible. BMI was categorised, primarily according to WHO criteria, into underweight, normal weight, overweight and obese (WHO, 1995). As there were only two subjects who were borderline underweight, BMI was later categorised into normal, overweight and obese for analysis. Therefore, the underweight women were included in the "normal" category.

Data for education was collected as years of schooling and, therefore, was categorised as follows: low (≤10 yr), medium (11-14) and high (15+) years of schooling. Menopausal status of the women were categorised according to WHO criteria as discussed previously in section 4.1.1 (WHO, 1981).

Categorical variables of interest were organised differently according to needs of analysis. For example, as there are very few perimenopausal women in the groups and their hormone levels were similar to postmenopausal women, the perimenopausal and postmenopausal status were merged together and menopausal status collapsed into two groups – premenopausal and postmenopausal To examine any confounding effect of religion, further analyses was done by grouping sedentees into Hindus and Muslims to examine differences in socio-economic and reproductive variables (see Table 5.1.10).

To evaluate effects of the timing of changes in the environment during development, CHI were further analysed on the basis of the stage of development they had reached when entering the UK, namely: 1) infancy and childhood (<8 yrs.) and peri-menarcheal (9-16). These categories roughly correspond with stages of neuro-endocrine development related to growth and maturation such as adrenarche, which typically occurs around year 8, and thus, may be relevant to the development of ovarian function (Karlerberg et al., 1994).

As mentioned previously, this study is part of a bigger, on-going project, so an exploratory analysis was done initially to examine the representativeness of my study to the main project. Characteristics that were examined are socio-demographic and

socio-economic, and menopausal status. Further comparisons between this study (Study Sample) and the main study (Main Sample) for reproductive variables such as age at menarche and age at menopause were done. Descriptive statistics for the study sample were performed through examining the distribution of the all variables of interests and comparing the means of the variables. Finally the means of the hormonal levels were compared.

As previously mentioned, the hormonal data were not normally distributed and were normalised by logarithmic transformation ($\log_{10}$). Therefore, the computed means are geometric.

Statistical analyses were performed by the following steps. To compare categorical variables, Chi square tests were performed, while to compare means of continuous variables independent sample t-tests or ANOVA were performed. Association of study groups with age group, nutritional status (BMI category), menopausal status, parasitic infections and worm infestations were examined by Chi-square tests. Tests also were done to examine differences in socio-economic condition and level of educational status between the groups. To find out any confounding factors that influence differences between SYL and

ADU, analysis was performed between two religious groups - Hindus and Muslims - within the SYL group.

To examine difference of hormonal indices between groups was performed by univariate analysis (GLM) after controlling for age. For testing hypotheses, each hormone data was analysed with standard multiple linear regression (MLR) to examine the effectiveness of the covariates to explain the hormone data and predict hormone levels by independent variables. Categorical variables with more than two categories that were used as predictor for MLR were reconstituted into dummy variables, for example, study group. All independent variables were checked for multicolinearity by Variance Inflation Factor (VIF) and tolerance statistics. The independent variables were excluded from the model where VIF was 10 or more and tolerance statistics below 0.2. For example, when height, weight and BMI were entered into same model, multicolinearity was detected between BMI and weight. Therefore, weight was excluded from the models.

For all analyses, levels of significance were set to $p<0.05$ as significant and $<.001$ as highly significant. Data were entered and analysed using Statistical Package for the Social Sciences (SPSS v.17.0).

CHAPTER 5

RESULTS

This chapter provides the results of the hormonal data collected for this study to assess ovarian reserve. Since it is part of a larger, ongoing, bio-cultural study of reproductive ageing and menopause, some variables (with a larger sample size) were collected with several other objectives. The study was carried out on four groups that include sedentee Bangladeshi women who grew up in Sylhet, NE Bangladesh and still live there (SYL), migrant Bangladeshi women who grew up in Bangladesh but migrated to the UK during adult life (ADU), Bangladeshi women who migrated to the UK during childhood (CHI), and white women of European origin who grew up in the UK (EUR).

The first section provides exploratory analyses of the characteristics of the sample population who gave blood to determine its representativeness against the larger main sample in case there were any inadvertent biases. As most of the women of CHI group gave blood for the hormone study, they were excluded from these comparisons. Therefore, this section provides the exploratory analysis for SYL, ADU and EUR groups. Those who did give blood samples will henceforth be called the

"*Study Sample*" while the entire sample will be referred to as the "*Main Sample*".

The second section reveals the descriptive analyses of the Study Sample across all the included groups. It examines the association of bio-characteristics and socio-economic characteristics of the women between the groups and identifies any factors influencing these associations. The third section describes general hormonal levels across the groups, and across age categories. In the fourth section, analyses are performed to test the specific hypotheses outlined earlier.

5.1 Exploratory analyses to examine representativeness of the Study Sample to the Main Sample

A total of 540 women from SYL, ADU, CHI and EUR participated in the Main Study of whom 207 women gave blood. About 29%, 33% and 34% SYL, ADU and EUR respectively of the main sample gave blood for hormone study, while 96% of CHI women participated in the hormone study (Table 5.1.1). It was because they were harder to recruit, working and less easy to access.

Table 5.1.1 Distribution of the women between Main Sample and Study Sample by study group

Group	Main Sample		Study Sample	
	N	%	N	%
SYL	157	100	45	29
ADU	174	100	57	33
CHI	55	100	53	96
EUR	154	100	52	34
Total	540	100	207	38

Note: SYL= women who grew up in Bangladesh and still living there; ADU= grew up in Bangladesh and migrated to the UK during adult life; CHI= Bangladeshi women migrated to the UK as child and grew up in the UK; EUR= white women of European origin in the UK.

Mean ages of the women are similar between the Main Sample (47.0 ± 0.32) and Study Sample (46.5 ±0.56) and also between the Main and Study Samples across the groups (SYL – 46.8 ± 0.57 vs 46.6 ± 1.03; ADU – 46.4 ± 0.55 vs 45.6 ± 0.92; and EUR – 47.9 ± 0.55 vs 47.4 ± 0.96; Table 5.1.2). The proportion of people in 5-year age categories between the Main and Study Sample is also very similar with no significant differences between any of these categories (Table 5.1.3).

Table 5.1.2 Mean age between groups by Main Sample and Study Sample

Variable	Sample population	Group											
		Total Sample			SYL			ADU			EUR		
		N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
Age	Study Sample	154	46.5	0.56	45	46.6	1.03	57	45.6	.92	52	47.4	0.96
	Main Sample	485	47.0	0.32	157	46.8	.57	174	46.4	.55	154	47.9	.55

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK

Table 5.1.3 Distribution of age category by the Main Sample and Study Sample across the groups

Variables (n (%))		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Age category	35-39	32 (21)	95 (20)	8 (18)	30 (19)	16 (28)	41 (24)	8 (15)	24(16)
	40-44	36 (23)	96 (20)	12 (26)	35 (22)	14 (25)	36 (21)	10 (19)	25 (16)
	45-49	30 (20)	104 (21)	7(16)	31 (20)	10 (17)	34 (20)	13 (26)	39 (25)
	50-54	29 (19)	100 (20)	10 (22)	31 (20)	9 (16)	33 (19)	10 (19)	36 (23)
	55-59	27 (17)	90 (19)	8 (18)	30 (19)	8 (14)	30 (16)	11 (21)	30 (20)
	Total	154 (100)	485 (100)	45 (100)	157 (100)	57 (100)	174 (100)	52 (100)	154 (100)

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK

Results of self-reported age at menarche suggest no significant difference between the Main Sample (13.0 ±.07) and Study Sample (13.1 ±0.13) and also consistent across the groups (SYL – 13.2 ± 0.11 vs 13.1 ± 0.23, ADU – 13.0 ± 0.10 vs 13.3 ± 0.18 and EUR – 12.8 ± 0.16 vs 12.8 ± 0.23) (Table 5.1.4).

Self-reported age at menopause of women between the Main (47.2 ± 0.29) and Study (47.4 ± 0.58) Samples are also comparable as well as between the Main and Study Samples within the groups (EUR – 49.2 ± 0.57 vs 50.0 ± 1.16, ADU – 47.5 ± 0.41 vs 47.1 ± 1.03 and SYL – 45.8 ± 0.44 vs 46.0 ± 0.75) (Table 5.1.4).

Results for the distribution of menopausal status are shown in Table 5.1.5. They suggest that more than half the women in the Main Sample and the Study Sample are pre-menopausal and about one third are post-menopausal. In SYL, there are 51% and 44% postmenopausal women in Study Sample and Main Sample respectively, while in ADU, there are 37% and 30% women in Study sample and Main Sample respectively. On the other hand, 29% and 30% of the EUR women are postmenopausal in the Study Sample and Main

Sample respectively. These differences, however, are not significant.

Table 5.1.4 Mean age at menarche and age at menopause between groups by Main and Study Sample

Variable	Sample population	Group											
		Total Sample			SYL			ADU			EUR		
		N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
Age at menarche	Study Sample	146	13.1	.12	45	13.1	.23	49	13.3	.18	52	12.8	.23
	Main Sample	476	13.0	.07	157	13.2	.11	165	13.0	.10	154	12.8	.16
Age at menopause	Study Sample	55	47.4	.58	23	46.0	.75	17	47.1	1.03	15	50.0	1.16
	Main Sample	179	47.2	.29	69	45.8	.44	65	47.5	.41	45	49.2	.57

Note: SYL= women who grew up in Bangladesh and still living there; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK

Table 5.1.5 Distribution of menopausal status by the Main and Study Sample across the groups

Variables (n (%))		Study population							
		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Menopausal status	Premenopausal	87 (56)	265 (55)	21 (47)	78 (50)	35 (61)	96 (55)	31 (60)	91 (60)
	Perimenopausal	12 (8)	39 (8)	1 (2)	10 (6)	5 (9)	13 (8)	6 (11)	16 (10)
	Postmenopausal	55 (36)	179 (37)	23 (51)	69 (44)	17 (30)	65 (37)	15 (29)	45 (30)
	Total	154(100)	483 (100)	45 (100)	157(100)	57 (100)	174(100)	52 (100)	152(100)

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK

There is no significant difference in mean BMI between the Main Sample and Study Sample for EUR (25.47 ± 0.39 vs 25.57 ± 0.70 respectively) and ADU (27.30 ± 0.24 vs 27.07 ± 0.44 respectively). However, for SYL the mean BMI of the Study Sample (24.89 ± 0.69) was found to be significantly lower (independent t-test: $t_{68.73}$= 2.304; $p<0.05$) compared to the Main Sample (26.16 ± 0.36), suggesting women who had a lower BMI in Sylhet participated more in the hormone study (Table 5.1.6).

When using weight status (i.e., BMI categorised as normal weight (<24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (>30 kg/m2), as shown in Table 5.1.7, there is no significant variation in weight status between the Study Sample and Main Sample. In the Study Sample, 43%, 39% and 17% are normal weight, overweight and obese respectively, while, in main sample, it is 38%, 45% and 17% respectively. There are no significant differences in weight categories between women in the Main Sample and the Study Sample for both ADU and EUR, but there is a significant (χ^2=9.02, df=3, $p<0.05$) difference for SYL; about half of the Sylheti women in the Study Sample are of normal weight compared to about one-third (34.4%) of women in the Main Sample.

Table 5.1.6 Mean BMI between groups by Main and Study Sample

Variable	Sample population	Group											
		Total Sample			SYL			ADU			EUR		
		N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
BMI†	Study Sample	154	25.93	0.36	45	24.89	0.69	57	27.07	0.44	52	25.57	0.70
	Main Sample	482	26.34	0.19	157	26.16	0.33	171	27.30	.24	154	25.47	0.39

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK

†Mean BMI of Study Sample significantly different from main sample in SYL (Independent T-test: $t_{68.73} = 2.304$; $p<0.05$*)

Table 5.1.7 Distribution of weight status by the Main and Study Sample across the groups

Variables (n (%))		Study population							
		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Weight status*	Normal	67 (43.5)	183 (38)	23 (51.1)	54 (34.4)	16 (28.1)	44 (25.8)	28 (53.8)	85 (55.2)
	Overweight	60 (39.0)	218 (45.2)	16 (35.6)	84 (53.5)	30 (52.6)	90 (52.6)	14 (26.9)	44 (28.6)
	Obese	27 (17.5)	81 (16.8)	6 (13.3)	19 (12.1)	11 (19.3)	37(21.6)	10 (19.3)	25 (16.2)
	Total	154 (100)	482 (100)	45 (100)	157 (100)	57 (100)	171 (100)	52 (100)	154 (100)

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK
Figures in parenthesises are percentage
* SYL: between Study Sample & Main Sample (Chi square=9.02, df=3, p<0.5)

Results for perceived financial condition suggest that, overall, a higher percentage of the Study Sample (34%) reported *struggling* compared to the Main Sample (25%), while a smaller percentage of women in the Study Sample perceived themselves to be *comfortable* (20%) or *well off* (5%) compared to the Main Sample (24% and 9% respectively) (Table 5.1.8). The difference in perceived financial condition between groups is significant (χ^2= 11.76, df = 3, p<0.01), which suggests that women who were economically vulnerable participated more in the hormone study. The distribution of perceived current financial condition of women across the groups reveals that this is homogenous between the Study and Main Sample for both EUR and ADU, but the distribution is significantly (χ^2=19.06 df=3, p<0.001) different in SYL. This result suggests that about three-fourths of sedentees in the Study Sample perceived their financial condition as either *struggling* (23%) or *OK* (51%) compared to 11% and 38% respectively in Main Sample. On the other hand, 19% and 7% of SYL perceived themselves to be *comfortable* and *well off* respectively in the Study Sample, while 30% and 21% reported respectively in the Main Sample (Table 5.1.8). Therefore, for the sedentees, financially deprived women participated more in the hormone study.

Level of education was categorised by *low* (< 10 years), *medium* (11 to 14 years) and *high* (> 15 years of schooling). There are no significant differences between the Main Sample and Study Sample, and the level of education across groups is distributed homogeneously between both samples (Table 5.1.9).

Table 5.1.8 Distribution of financial condition by the Main and Study Sample across the groups

Variables (n(%))		Study Population							
		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Financial Condition[1, 2]	Struggling	51 (33.6)	122 (25.4)	10 (23.3)	17(11.0)	28 (49.1)	67 (38.5)	13 (25)	38 (25.0)
	OK	63 (41.4)	199 (41.3)	22 (51.1)	59 (38.0)	18 (31.6)	64 (36.8)	23 (44.2)	76 (50.5)
	Comfortable	31 (20.4)	118 (24.5)	8 (18.6)	46 (29.7)	10 (17.5)	38 (21.8)	13 (25)	34 (22.4)
	Well off	7 (4.6)	42 (8.8)	3 (7.0)	33 (21.3)	1 (1.8)	5 (2.9)	3 (5.8)	4 (2.6)
	Total	152 (100)	481 (100)	43 (100)	155 (100)	57 (100)	174 (100)	52 (100)	152 (100)

Note: SYL= women who grew up in Bangladesh and still living there ; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK
Figures in parenthesises are percentage
1. Between Study Sample and Main Sample of Total: (Chi square=10.286, df=3, $p<0.05$)
2. Between Study Sample and Main Sample of Sedentees: (chi square =19.06, df =3, $p<0.001$)

Table 5.1.9 Distribution of education levels by the Main and Study Sample across the groups

Variables (N (%))		Study Population							
		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Educational level	Low	86 (55.8)	292 (60.2)	31 (68.9)	102 (65)	43 (75.4)	147 (84.5)	12 (23.0)	43 (27.9)
	Medium	41 (26.6)	116 (23.9)	12 (26.7)	48 (30.5)	9 (15.8)	19 (10.9)	20 (38.5)	49 (31.8)
	High	27 (17.6)	77 (15.9)	2 (4.4)	7 (4.5)	5 (8.8)	8 (4.6)	20 (38.5)	62 (40.3)
	Total	154 (100)	485 (100)	45 (100)	157 (100)	57 (100)	174 (100)	52 (100)	154 (100)

Note: SYL= women who grew up in Bangladesh and still living there; ADU= grew up in Bangladesh and migrated to the UK during adult life; EUR= women grew up as European in the UK
Figures in parenthesises are percentage

Very few EUR women disclosed their religious faith, while all the migrant women declared themselves as Muslims and sedentees responded as either Muslims or Hindus (Table 5.1.10). Overall, only 339 women responded to the question about religion and of these 87%, 10% and 3% answered as Muslim, Hindu and others (i.e. Christian, Buddhist and other religious group) respectively in the Main Sample, compared to 78%, 15% and 7% respectively in Study Sample. On the other hand, more than one third (36%) of SYL in the Study Sample are Hindu, compared to only 22% in the Main Sample. In fact, out of a total of 34 Hindu women in the Main Sample, 16 (47%) gave blood, while only 27 (23%) of the 120 Muslim women in the Main Sample contributed blood.

Table 5.1.10 Distribution of religion by the Main and Study Sample across the groups

Variables (N (%))		Study Population							
		Total		SYL		ADU		EUR	
		Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample	Study Sample	Main Sample
Religion	Muslim	84 (77.8)	294 (86.7)	27 (61.4)	120 (77.4)	57 (100)	174 (100)		
	Hindu	16 (14.8)	34(10.0)	16 (36.4)	34 (21.9)				
	Other	8 (7.4)	11 (3.3)	1 (2.2)	1 (0.7)			7 (100)	10 (100)
	Total	108 (100)	339 (100)	44 (100)	155 (100)	57 (100)	174 (100)	7(100)	10 (100)

Note: EUR= women grew up as European in the UK; ADU= grew up in Bangladesh and migrated to the UK during adult life; SYL= women who grew up in Bangladesh and still living there

5.2 Descriptive analyses of the Study Sample

This section reveals the descriptive analyses of the Study Sample across the groups including bio-characteristics such as mean ages, age categories, age at menarche, age at menopause, menopausal status, BMI, height, weight, parasitic infections including helminths, and socio-economic characteristics such as education levels, and perceived current financial conditions

A total of 203 blood samples of the women were tested for inhibin B, anti-müllerian hormone (AMH) and follicle stimulating hormone (FSH), of whom 45 (22%) are SYL, 57 (28%) are ADU, 49 (24%) are CHI and 52 (26%) are EUR (table 5.2.1).

The mean ages of the women across groups are comparable between the EUR (47.4 ± SEM 0.96), ADU (45.6 ± 0.92) and SYL (46.6 ± 1.03), but CHI (39.3 ± 0.50) had younger women due to their more recent history of migration (Figure 5.2.1). The distribution by age category is depicted in table 5.2.2.

Table 5.2.1 Distribution of Study Sample by group

Study Group	N	Percentage
Sedentee Bangladeshi (SYL)	45	22
Adult migrant Bangladeshi (ADU)	57	28
Child migrant Bangladeshi (CHI)	49	24
Women of European Descent (EUR)	52	26
Total	203	100

Figure 5.2.1 Mean age of the women by study group

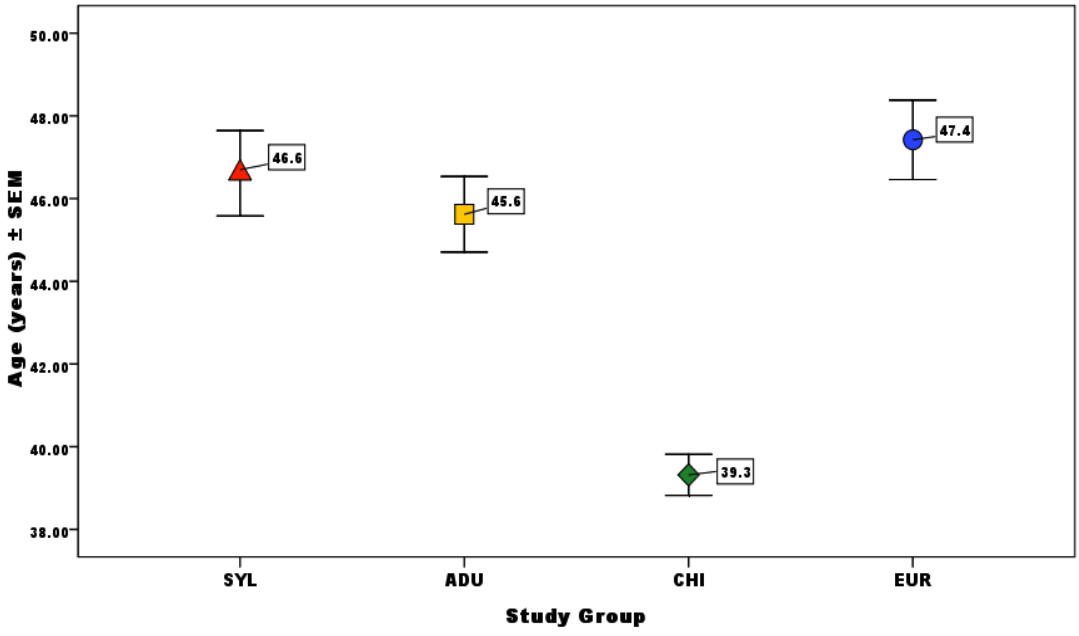


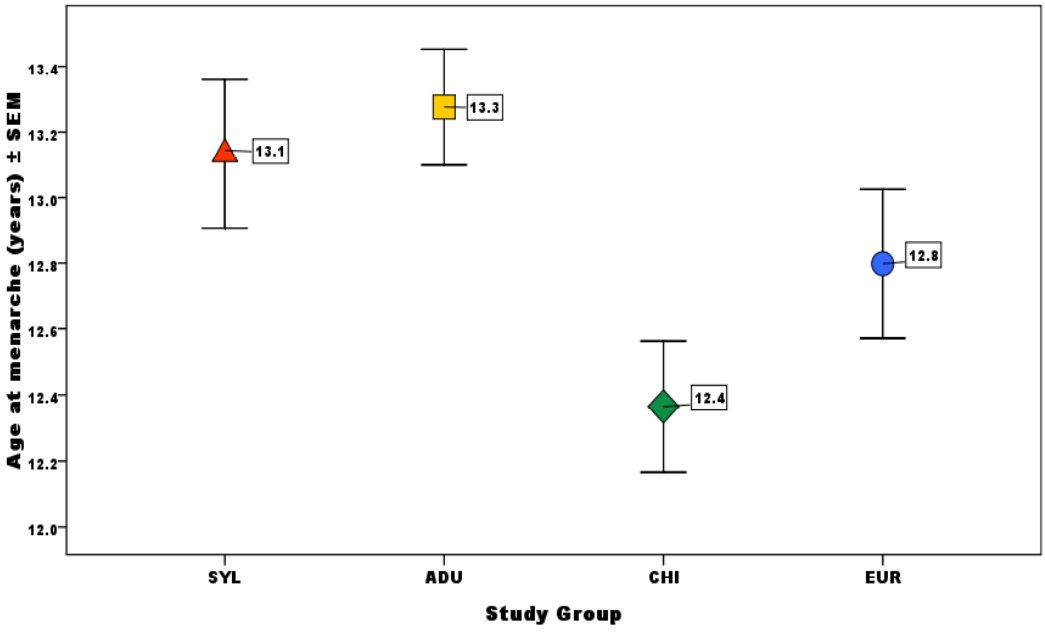
Table 5.2.2 Distribution of age category of the women by study group

Age category	Study Group				
	SYL	ADU	CHI	EUR	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
35-39	8 (18)	16 (28)	30 (61)	8 (16)	62 (30)
40-44	12 (27)	14 (25)	13 (27)	11 (21)	50 (25)
45-49	7 (15)	10 (17)	6 (12)	13 (25)	36 (18)
50-54	10 (22)	8 (14)		9 (17)	27 (13)
55-59	8 (18)	9 (16)		11 (21)	28 (14)
Total	45 (100)	57 (100)	49(100)	52 (100)	203 (100)

Chi square= 43.124, df= 16, $p<0.001$ (all groups)
Chi square= 7.691, df = 6, $p>0.05$, ns (SYL, ADU & EUR)

Results for recalled age at menarche in women suggests that EUR (12.8 ± SEM 0.23) and CHI (12.4 ± 0.20) have an earlier age at menarche compared to ADU (13.3 ± 0.18) and SYL (13.1 ± 0.23) (Figure 5.2.2). The means are significantly different between groups (ANOVA; $F_{3, 190}$ =3.746, p<0.05) while a post hoc tests suggests that the mean age at menarche is significantly different between ADU and CHI (p<0.01), and SYL and CHI (p<0.05).

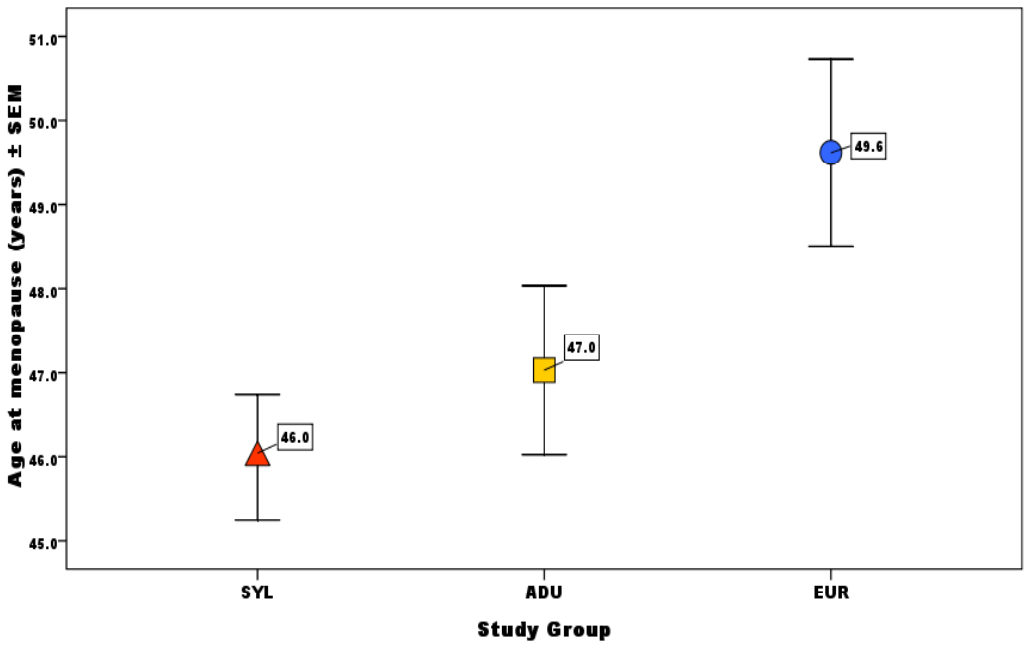
Figure 5.2.2 Mean age at menarche of the women by study group



$F_{3,190}$ =3.746, p<0.05; CHI<ADU,P<0.01

Recalled mean age at menopause in the postmenopausal women (Figure 5.2.3) suggests that EUR (49.6 ± SEM 1.11) have a later age at menopause compared to ADU (47.0 ± 1.0), and SYL (46.0 ± 0.75). However, CHI has only one post-menopausal woman. Therefore, analyses were carried out only between SYL, ADU and EUR and reveals that there are significant differences in age at menopause between groups (ANOVA; $F_{2,\ 52}$= 3.848, $p<0.05$). A *Post-hoc test* reveals that SYL have a significantly ($p<0.01$) lower self-reported mean age at menopause compared to EUR.

Figure 5.2.3 Mean age at menopause of the menopausal women by study group



$F_{2,52}$ =3.848, p< 0.05; SYL<EUR, P<0.05

For menopausal status, the women of each group were grouped into premenopausal, perimenopausal and postmenopausal according to their self-reported menopausal status (Table 5.2.3). Despite matching the groups by age-categories during recruitment, there is a significant difference (chi-square = 38.807, df = 6, $p<0.001$)) in menopausal status between SYL, ADU, CHI and EUR, while menopausal status is not different between SYL, ADU and EUR. However, only one woman in CHI group is postmenopausal, while none of the women in this group is perimenopausal. The result also suggests that there is a lower proportion of pre-menopausal SYL women (47%) in comparison to more than half the EUR (60%) and ADU (61%) groups. On the other hand, SYL (51%) has the highest percentage of post-menopausal women while both EUR and ADU have about 30% in this category. However, there are 12% and 9% perimenopausal women in EUR and ADU respectively, while SYL has only 1 (2.2%) perimenopausal women.

Further analyses on menopausal status were performed between EUR, ADU and SYL, as almost all the women in CHI are premenopausal. The results shown in figure 5.2.4 suggest that overall SYL women reach menopause earlier than EUR, and this is as low as 42.45 years. In the 40-44 year age group, SYL have the highest percentage of women (17%) who reached menopause

compared to only 8% of ADU; none of the EUR reached menopause in that age category. Moreover, none of the women in the 50-55 year age category among SYL or ADU are pre-menopausal compared to 33% premenopausal women among EUR in that age category.

Table 5.2.3 Distribution of menopausal status of the women by study group

Menopausal status (N (%))	Study Group			
	SYL	**ADU**	**CHI**	**EUR**
Premenopausal	21 (47)	35 (61)	48 (98)	31 (60)
Perimenopausal	1 (2)	5 (9)		6 (11)
Postmenopausal	23 (51)	17 (30)	1 (2)	15 (29)
Total	45 (100)	57 (100)	49 (100)	52 (100)

chi-Square = 38.8.07, df = 6, p< 0.001 (all group)
chi square= 8.128, df=4, p>0.05 (ns) (SYL, ADU AND EUR)

Figure 5.2.4 Distribution of the women by age across the menopausal status among the groups

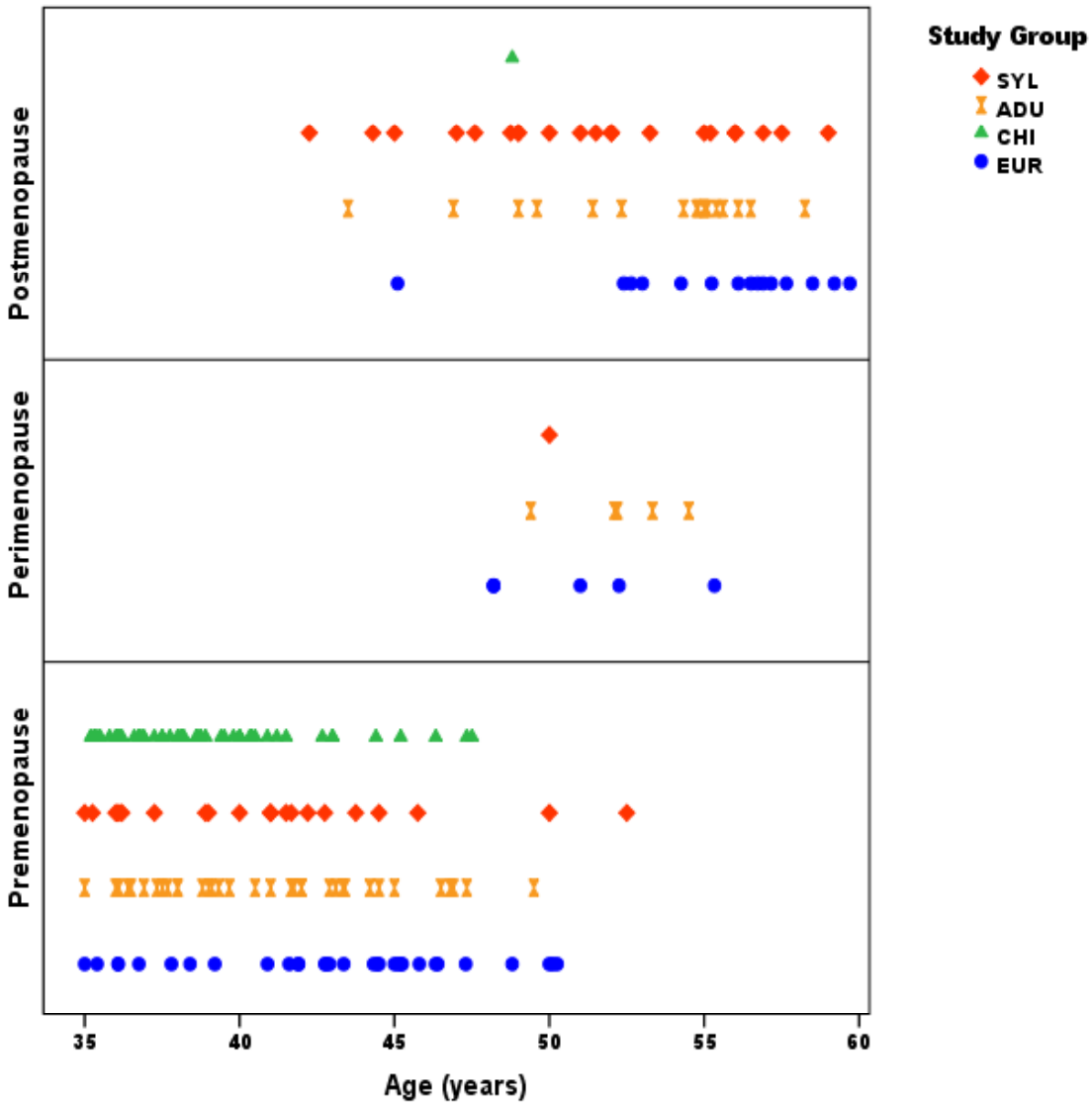


Table 5.2.4 shows the mean number of children, age at first child and age at last child. Analysis of variance reveals that parity is significantly (ANOVA; $F_{3, 171=}21.53$, $p<.001$) different between groups. A *Post hoc* test suggests that women in EUR group have a significantly lower number of children compared to women in SYL, ADU and CHI, while there is no significant difference between the 0numbers of children of the women of three Bangladeshi (SYL, ADU & CHI) groups. Mean age at first child (ANOVA; $F_{3, 170}=2.58$, p=ns) and mean age at last child ($F_{3,161}=1.26$, p=ns) are not significantly different between groups.

Table 5.2.4 Distribution of the women according to reproductive history by study group

Variable	SYL (n=45)		ADU (n=54)		CHI (n=47)		EUR (n=39)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Number of Children[1]	2.9	0.22	3.6	0.21	3.9	0.68	1.4	0.24
Age at first child[2]	21.2	0.67	21.2	0.73	20.6	0.65	24.9	1.25
Age at last child[3]	30.0	0.56	31.8	0.55	31.0	0.56	31.7	2.00

1. $F_{3, 181}=7.750$, p<.001; EUR<CHI, ADU & SYL <.05
2. $F_{3, 180}=2.150$, p=ns
3. $F_{3,170}=1.636$, p=ns

Mean height, weight and BMI of the women by groups are depicted in the table 5.2.5. The Mean height is significantly (ANOVA; $F_{3,199}$=35.066, p<.001) different between groups. *Posthoc* test suggests that EUR women are significantly taller than SYL, ADU and CHI, while there is no significant difference in height between Bangladeshi women (SYL, ADU and CHI). On the other hand, mean weight is significantly (ANOVA; $F_{3,199}$=7.728, p<.001) different between groups. SYL has significantly lower mean weight than the other three groups, while EUR, CHI and ADU do not have significantly different weights.

Mean BMI is significantly (ANOVA; $F_{3, 199}$ = 3.967, p<0.005; Table 5.2.5) different between groups with a higher mean BMI in ADU (27.07 ± SEM 0.44) and CHI (27.48 ± 0.59) compared to EUR (25.57 ± 0.70), and SYL (24.89 ± 0.69). *Post-hoc* tests reveal that there are significant differences between SYL and ADU (p<0.01), SYL and CHI (p<0.01) and EUR and CHI (p<0.05) in mean BMI.

Weight status (BMI category) is found to be significantly different (chi square=19.069, df=8, p<0.05) between groups (Table 5.2.6). As more than half of the EUR (54%) and SYL (51%) are of normal weight compared to about 28% and 29% of ADU and CHI respectively. On the other hand, more than two third of

the ADU (72%) and CHI (71%) are overweight to obese compared to EUR (46%) and SYL (49%). However, comparing the obese women between the groups, CHI has the highest (29%) percentage of obese women followed by EUR (19%) and ADU (19%), while SYL has lowest (13%).

Table 5.2.5 Height, weight and BMI of the women by the study group

Variable	SYL			ADU			CHI			EUR		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
Height (cm)*	45	150.6	0.83	57	153.1	0.93	49	154.1	0.88	52	162.5	0.80
Weight (kg)‡	45	56.6	1.73	57	63.6	1.35	49	65.3	1.51	52	67.7	1.99
BMI (kg/m²)†	45	24.89	0.69	57	27.07	0.44	49	27.49	0.59	52	25.57	0.70

*$F_{3,199}$=35.066, p<.001 ; EUR>SYL, ADU & CHI < 0.001
‡ $F_{3,199}$=7.728, p<.001 ; SYL < EUR, CHI & ADU < .05
†$F_{3,199}$= 3.967, p<0.005; SYL<ADU; SYL<CH, EUR<CHI

Table 5.2.6 Distribution of weight status of the women by study group

Weight status (N (%))	Study Group				Total
	SYL	ADU	CHI	EUR	
Normal	23 (51)	16 (28)	14 (29)	28 (54)	81 (40)
Overweight	16 (36)	30 (53)	20 (42)	14 (27)	80 (40)
Obese	6 (13)	11 (19)	14(29)	10 (19)	41 (20)
Total	45 (100)	57(100)	48 (100)	52 (100)	202 (100)

chi square=19.066, df=8, $p<0.05$ (All group),

chi square= 11.852, df= 4, $p<0.05$ (SYL, ADU & EUR)

Levels of education are categorised on the basis of numbers of years attending school: *Low* (0-10 years), *Medium* (11-14 years) and *High* (>14 years). Levels of education are significantly different (chi square= 61.162 df= 6, p<0.001*) across groups. More than two-fifths (43%) of CHI, two-thirds (69%) of SYL and three-quarters (75%) of ADU have low levels of education compared to only 23% of EUR. On the other hand, more than one-third of EUR (39%) have a high level of education, compared to ADU (9%), SYL (4%), and CHI (2.1%). More than half of CHI is educated to medium level, compared to 39%, 27%, and 16% of EUR, SYL and ADU women respectively (Table 5.2.7).

There are significant differences between groups in perceived financial condition (chi square = 22.638, df =9, p<0.01) (Table 5.2.8). The results suggest that 25% and 44% of EUR perceived their current financial condition as struggling and OK respectively, compared to 23% and 51% respectively in SYL, 49% and 31% respectively in ADU, and 11% and 57% respectively in CHI. However, overall one-third or less of women across the groups perceived that their financial condition was either comfortable or well-off, although a slightly higher proportion of EUR (31%) and CHI (32%) perceived their current condition as "comfortable" to "well-off" compared to ADU (19%) and SYL (26%).

Table 5.2.7 Distribution of level of education of the women by the study group

Level of education (N (%)	Study Group				Total
	SYL	ADU	CHI	EUR	
Low	31 (68.9)	43 (75.4)	21 (43.7)	12 (23.1)	107 (53.0)
Medium	12 (26.7)	9 (15.8)	26 (54.2)	20 (38.5)	68 (33.7)
High	2 (4.4)	5 (8.8)	1 (2.1)	20 (38.5)	27 (13.4)
Total	45 (100)	57 (100)	48 (100)	52 (100)	202 (100)

chi square= 61.162 df= 6, p<0.001*

Table 5.2.8 Distribution of perceived current financial situation of the women by the study group

Current financial situation (N (%))	Study Group				Total
	SYL	ADU	CHI	EUR	
Struggling	10 (23.3)	28 (49.1)	5 (10.6)	13 (25.0)	56 (28.0)
OK	22 (51.2)	18 (31.6)	28 (57.4)	23 (44.2)	91 (45.5)
Comfortable	8 (18.5)	10 (17.5)	13 (27.7)	13 (25.0)	44 (22.0)
Well off	3 (7.0)	1 (1.8)	2 (4.3)	3 (5.8)	9 (4.5)
Total	43 (100)	57 (100)	48 (100)	52 (100)	200 (100)

chi square = 22.638, df=9, $p<0.01$*

Table 5.2.9 shows the distribution of helminthic infestion (pin worm, round worm, hook worm and whip worm) and parasitic infections (malaria, leishmaniasis, amoebic dysentery) across the groups. Results for helminthic infestation suggests that a larger proportion of the Bangladeshi women, both ADU (72%) and SYL (84%) have been infested by helminths, which is significantly higher (chi square= 70.074, df= 3, $p<0.001$) than EUR (8%) and CHI (29%). When the women were asked about whether they had ever been infected with parasites, a significantly higher (chi-square=17.626, df= 3, $p<0.001$) percentage of SYL (35%), CHI (17%) and ADU (17%) responded that they had experienced parasitic infection compared to EUR (2%).

Table 5.2.9 Distribution of intestinal worm infestation and parasitic infection of the women by the study group

Variables (N (%))		Study Group				Total
		SYL	ADU	CHI	EUR	
Helminthic infestation*	Yes	32 (84.2)	40 (72.7)	11 (28.9)	4 (8.2)	87 (48.3)
	No	6 (15.8)	15 (27.3)	27 (71.1)	45 (91.8)	93 (51.7)
	Total	38 (100)	55 (100)	38 (100)	49 (100)	180 (100)
Parasitic infection¥	No	28 (65.1)	44 (83.0)	34 (82.9)	49 (98.0)	155 (82.9)
	Yes	15 (34.9)	9 (17.0)	7 (17.1)	1 (2.0)	32 (17.1)
	Total	43 (100)	53 (100)	41 (100)	50 (100)	187 (100)

*Chi square = 70.074, df = 3, p<0.001
¥Chi square = 17.626, df= 3, p<0.001

5.2.2 Distribution of age category, education, nutritional status, financial condition and menopausal status of SYL by religion

Further analysis of the women in SYL group was performed to examine whether there are any differences between the Hindu and Muslim populations given the high proportion of Hindus in this group compared to none among the migrants. Comparing the mean age between Hindu and Muslim in SYL reveals that Hindus (48.6 ± 1.85) are older than Muslims on average (45.42 ± 1.17), but this difference is not significant. The distribution by age categories is shown in Table 5.2.7(a) and suggests that a larger proportion of Hindus are aged >45 years compared to Muslims, but there is no significant heterogeneity of the distribution of the women across the age categories between groups.

Mean BMI of the Muslim women (26.29 ± 0.74) is significantly (t_{41}=2.337, $p<0.05$) higher compared to Hindu (23.1 ± 1.26) women. A significantly higher (chi square= 6.989, df =2, $p<0.05$*) percentage of Muslim women are overweight or obese compared to Hindu women. Results for menopausal status show that the proportion of postmenopausal women in Hindus is twofold higher than premenopausal women in the same group (Table 5.2.7.a). On the other hand, Muslims are equally distributed between premenopausal and postmenopausal women. However,

the difference is not significant between Hindus and Muslims (Table 5.2.10.a).

Results for educational status suggests that Muslim women are more educated (low 63% vs 88%; medium 33% vs 13%, high 4% vs none) compared to Hindu, however the difference is not significant (Table 5.2.10.b). For perceived financial condition, 40% of Hindu women perceived their financial condition as *struggling* compared to only 15% of Muslim women (Table 5.2.10.b). On the other hand, none of the Hindu women perceive their financial condition as *well off*. Hindus in SYL of the Study Group are less affluent compare to Muslims although the differences are not significant.

These results suggest that Hindus in SYL might be economically, educationally and nutritionally different from Muslim in SYL in this study. Therefore Hindu women may lead to confounding in interpreting differences between SYL and ADU. However the sample size is too small for confirmation.

Table 5.2.10(a) Distribution of age category, weight status and menopausal status by religion of SYL group

Variables		Muslim		Hindu		Total	
		N	%	N	%	N	%
Age Category†	**35-39**	5	18.5	2	12.5	7	16.3
	40-44	9	33.3	3	18.8	12	27.9
	45-49	3	11.1	4	25.0	7	16.3
	50-54	8	29.6	2	12.5	10	23.3
	55-59	2	7.4	5	31.3	7	16.3
Weight Status‡	**Normal**	9	33.3	12	75.0	21	48.8
	Overweight	13	48.1	3	18.8	16	37.2
	Obese	5	18.5	1	6.3	6	14.0
Menopausal Status¥	**Premenopausal**	14	51.9	6	37.5	20	46.5
	Perimenopausal	1	3.7			1	2.3
	Postmenopausal	12	44.4	10	62.5	22	51.2

†chi square=6.956, df=4, p=0.138 (ns);
‡chi square=6.989, df=2, p<0.05*;
¥chi square=1.687, df= 2, p=0.595 (ns)

Table 5.2.10(b) Distribution of socio-economic and level of education by religion of SYL group

Variables		Muslim		Hindu		Total	
		N	%	N	%	N	%
Financial condition†	Struggling	4	15.4	6	40.0	10	24.4
	OK	14	53.8	6	40.0	20	48.8
	Comfortable	5	19.2	3	20.0	8	19.5
	Well off	3	11.5			3	7.3
Educational status‡	Low	17	63.0	14	87.5	31	72.1
	Medium	9	33.3	2	12.5	11	25.6
	High	1	3.7			1	2.3

†chi square=4.471, df =3, p=.229 (ns)
‡chi square=3.136, df =2, p = .208 (ns)

5.3 Analysis of hormone levels

This section describes hormone levels across the groups. As the hormone data were not normally distributed and were skewed, the data were transformed (log base 10). The transformed data were tested for assumption of normality and found symmetrically distributed. The transformed data were used for hormone level analyses. The geometric mean (GM) was also used for all mean hormonal analyses.

A total of 203 blood samples from women were tested for inhibin B, AMH and FSH. However, 11 women were excluded from data analyses as 5 were diabetic, 4 had polycystic ovarian syndrome and 2 had thyroid related problems. Therefore, a total of 192 women were included in hormone data analyses.

5.3.1 Distribution of hormone levels

Overall mean levels of inhibin B, AMH and FSH are depicted in table 5.3.1. The results show that mean inhibin B is highest in CHI (53.58 ± SEM 4.90) followed by EUR (23.30 ± 4.03) and ADU (20.07 ± 4.07), while SYL (15.91 ± 3.46) has the lowest. The overall mean AMH levels by groups also show that CHI (9.66± 2.17) is highest followed by EUR (5.70 ± 1.90) and ADU (1.57 ± 0.95), while SYL (1.19 ± 0.73) has the lowest AMH levels. Overall mean FSH levels show that SYL (27.49 ± 7.05) has the highest

followed by ADU (19.10 ± 4.33) and EUR (18.12 ± 5.53), while CHI (7.84 ± 1.02) have the lowest overall mean FSH.

As women in the CHI group are younger and mostly premenopausal compared to the other three groups (SYL, ADU and EUR), further analyses were done on the pre-menopausal women (Table 5.3.2). Mean inhibin B levels are highest in CHI (55.87 ± 4.81), followed by EUR (35.68 ± 5.15) and ADU (29.04 ± 5.63), while SYL (25.91 ± 5.71) has the lowest. Overall mean AMH levels by groups show that CHI (10.44± 2.13) has the highest followed by EUR (5.57 ± 2.75) and SYL (3.49 ± 1.19), while ADU (3.33 ± 1.31) has the lowest AMH levels. Overall mean FSH levels show that ADU (10.56 ± 3.26) has the highest followed by SYL (9.84 ± 2.86) and CHI (7.52 ± 0.38), while EUR (7.47 ± 1.21) has the lowest overall mean FSH.

The result of overall mean of inhibin B, AMH and FSH levels of premenopausal women, inhibin B and AMH were found to be higher among the CHI and EUR compared to SYL and ADU, while FSH levels were found to be higher among SYL and ADU compared to CHI and EUR (Table 5.3.2). Analysis of variance result suggested that mean inhibin B ($F_{3,\ 121}$=5.748, p<0.001), AMH ($F_{3,126}$=4.614, p<0.004) and FSH ($F_{3.127}$=3.013) levels are significantly different between groups. Post hoc tests suggested that CHI has significantly higher inhibin B (p<0.05) and AMH

($p<0.05$), while ADU has significantly higher FSH than CHI and EUR.

The result of overall mean of inhibin B, AMH and FSH levels by menopausal status shows that inhibin B and AMH levels of the perimenopausal and postmenopausal women are almost at minimum detection levels, while FSH levels are higher in all study groups (Table 5.3.3-5).

As the hormone levels of the women are dependent on their age, this comparison of the overall hormonal levels does not give the actual picture of variability of the hormonal levels. Therefore, comparing age-related hormonal levels gives a better conclusive idea about the variation of hormonal levels between groups.

Table 5.3.1 Distribution of mean hormone levels by study group

Study group	inhibin B (pg/ml)			AMH (pmol/L)			FSH (mIU/mL)		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	41	15.91	3.46	41	1.19	0.73	41	27.49	7.05
ADU	55	20.07	4.07	55	1.57	0.95	55	19.10	4.33
CHI	41	53.58	4.90	46	9.66	2.17	47	7.84	1.02
EUR	49	23.30	4.03	49	5.70	1.90	49	18.12	5.53

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.2 Distribution of mean hormone levels of the premenopausal women by the study group

Study group	inhibin B (pg/ml)[1]			AMH (pmol/L)[2]			FSH (mIU/mL)[3]		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	20	25.9	5.71	20	3.49	1.19	20	9.84	2.86
ADU	35	29.04	5.63	35	3.33	1.31	35	10.56	3.26
CHI	40	55.87	4.81	45	10.44	2.13	46	7.52	0.38
EUR	30	35.68	5.15	30	5.57	2.75	30	7.47	1.21

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means
1. $F_{3,\ 121}$=5.748, p<0.001, ADU & SYL<CHI
2. $F_{3,126}$=4.614, p<0.004, ADU & SYL<CHI
3. $F_{3.127}$=3.013, p<0.033, ADU> CHI & EUR

Table 5.3.3 Distribution of mean inhibin B (pg/ml) levels by menopausal status and study group

Study group	Premenopausal			Perimenopausal			Postmenopausal		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	20	25.9	5.71	1	10		20	10	
ADU	35	29.04	5.63	4	10		16	11.98	1.06
CHI	40	55.87	4.81						
EUR	30	35.68	5.15	5	14.62	5.24	14	11.04	1.40

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.4 Distribution of mean AMH (pmol/L) levels by menopausal status and group

Study group	Premenopausal			Perimenopausal			Postmenopausal		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	20	3.49	1.19	1	0.71		20	0.42	
ADU	35	3.33	1.31	4	0.42		16	0.42	
CHI	45	10.44	2.13				1	0.42	
EUR	30	5.57	2.75	5	0.42		14	0.42	

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.5 Distribution of mean FSH (mIU/mL) levels by menopausal status and group

Study group	Premenopausal			Perimenopausal			Postmenopausal		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	20	9.84	2.86	1	17.55		20	78.50	8.29
ADU	35	10.56	3.26	4	42.82	12.82	16	57.02	6.92
CHI	46	7.52	0.38				1	52.55	
EUR	30	7.47	1.21	5	70.97	18.09	14	74.22	5.99

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

5.3.2 Correlation between hormonal levels and age among the study population

Pearson's (r) correlation between hormone levels and age was examined (Table 5.3.6 and Figure 5.3.1-3). A highly significant negative correlation was found between inhibin B and age (one tail; r= -0.696; n=186; p<0.001). AMH and age are also significantly negatively correlated (r=-0.789; n=191; p<0.001). On the other hand, there is a significant positive correlation (r=0.794; n=192; p<0.001) between FSH and age. Results also show a significant positive correlation between inhibin B and AMH (r=0.782; n=186; p<0.001), but the correlations between inhibin B and FSH (r=-0.727; N=186; p<0.001), and AMH and FSH (r=-0.807; N=191; p<0.001) are found to be significantly negative.

Further analysis of Pearson's correlation between hormone levels and age among the premenopausal women were examined (Table 5.3.7 and Figure 5.3.4-6). A highly significant negative correlation was found between inhibin B and age (one tail; r= -0.434; n=129; p<0.001). AMH and age are also significantly negatively correlated (r=-0.609; n=134; p<0.001). On the other hand, there is a significant positive correlation (r=0.426; n=135; p<0.001) between FSH and age. Results also show a significant positive correlation between inhibin B and AMH (r=0.604; n=129;

$p<0.001$), but the correlations between inhibin B and FSH ($r=-0.469$; $N=129$; $p<0.001$), and AMH and FSH ($r=-0.613$; $N=134$; $p<0.001$) are found to be significantly negative.

All correlation analyses were performed on log transformed hormone values.

Table 5.3.6 Pearson's correlation matrix between hormones and age

Variables	Pearson	Age	Inhibin B	AMH
Inhibin B	Correlation Coefficient	-0.696**	--	--
	Sig. (1-tailed)	.000	--	--
	N	186	--	--
AMH	Correlation Coefficient	-0.789**	.782**	--
	Sig. (1-tailed)	.000	.000	--
	N	191	186	--
FSH	Correlation Coefficient	0.794**	-0.727**	-0.807**
	Sig. (1-tailed)	.000	.000	.000
	N	192	186	191

Note: Correlation analyses were performed on log-transformed hormone data

**. Correlation is significant at the 0.01 level (1-tailed).

Table 5.3.7 Pearson's correlation matrix between hormones and age of the premenopausal women

Variables	Pearson	Age	Inhibin B	AMH
Inhibin B	Correlation Coefficient	-0.434**	--	--
	Sig. (1-tailed)	.000	--	--
	N	129	--	--
AMH	Correlation Coefficient	-0.609**	.604**	--
	Sig. (1-tailed)	.000	.000	--
	N	134	129	--
FSH	Correlation Coefficient	0.426**	-0.469**	-0.613**
	Sig. (1-tailed)	.000	.000	.000
	N	135	129	134

Note: Correlation analyses were performed on log-transformed hormone data

**. Correlation is significant at the 0.01 level (1-tailed).

Figure 5.3.1 Correlation between inhibin B and age by study group

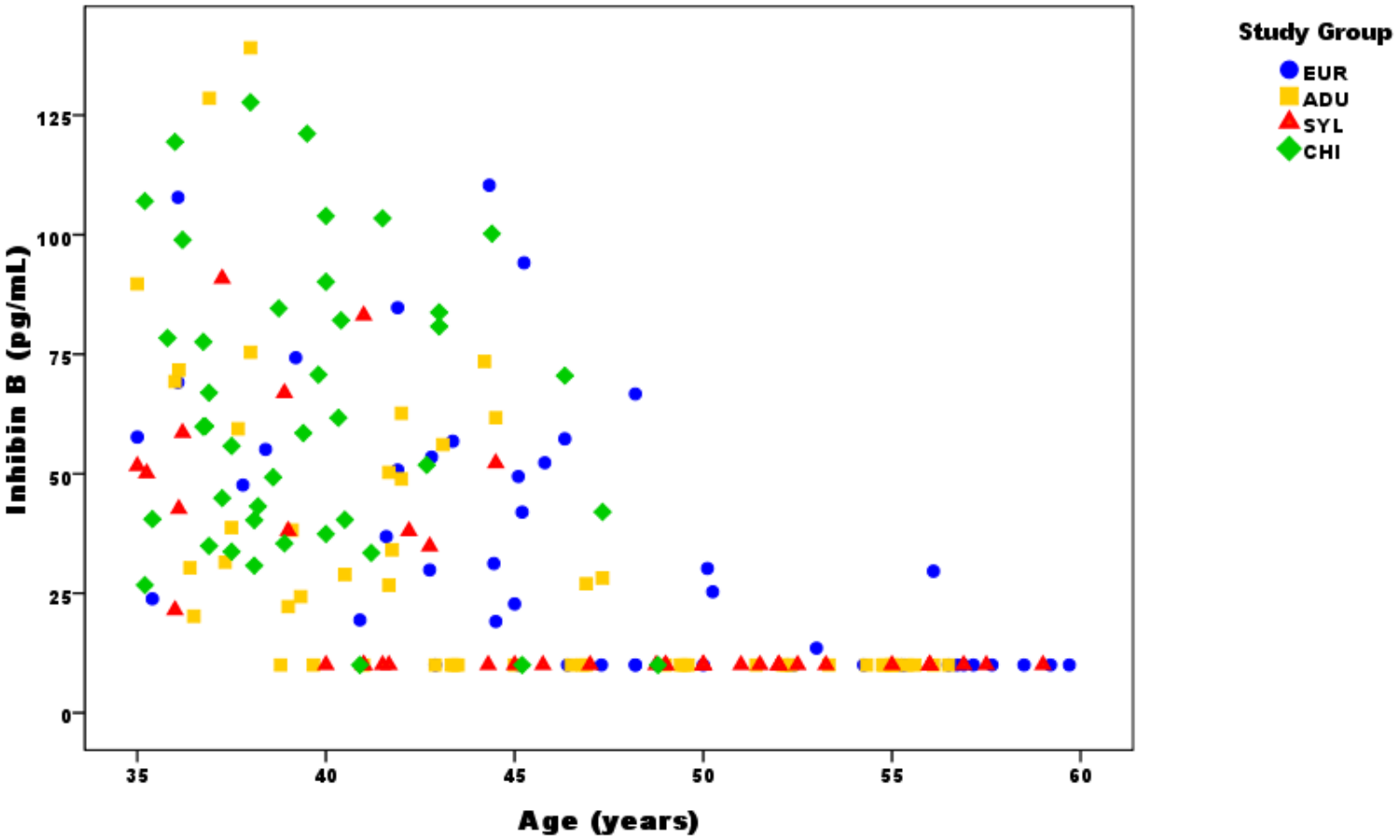


Figure 5.3.2 Correlation between AMH and age by study group

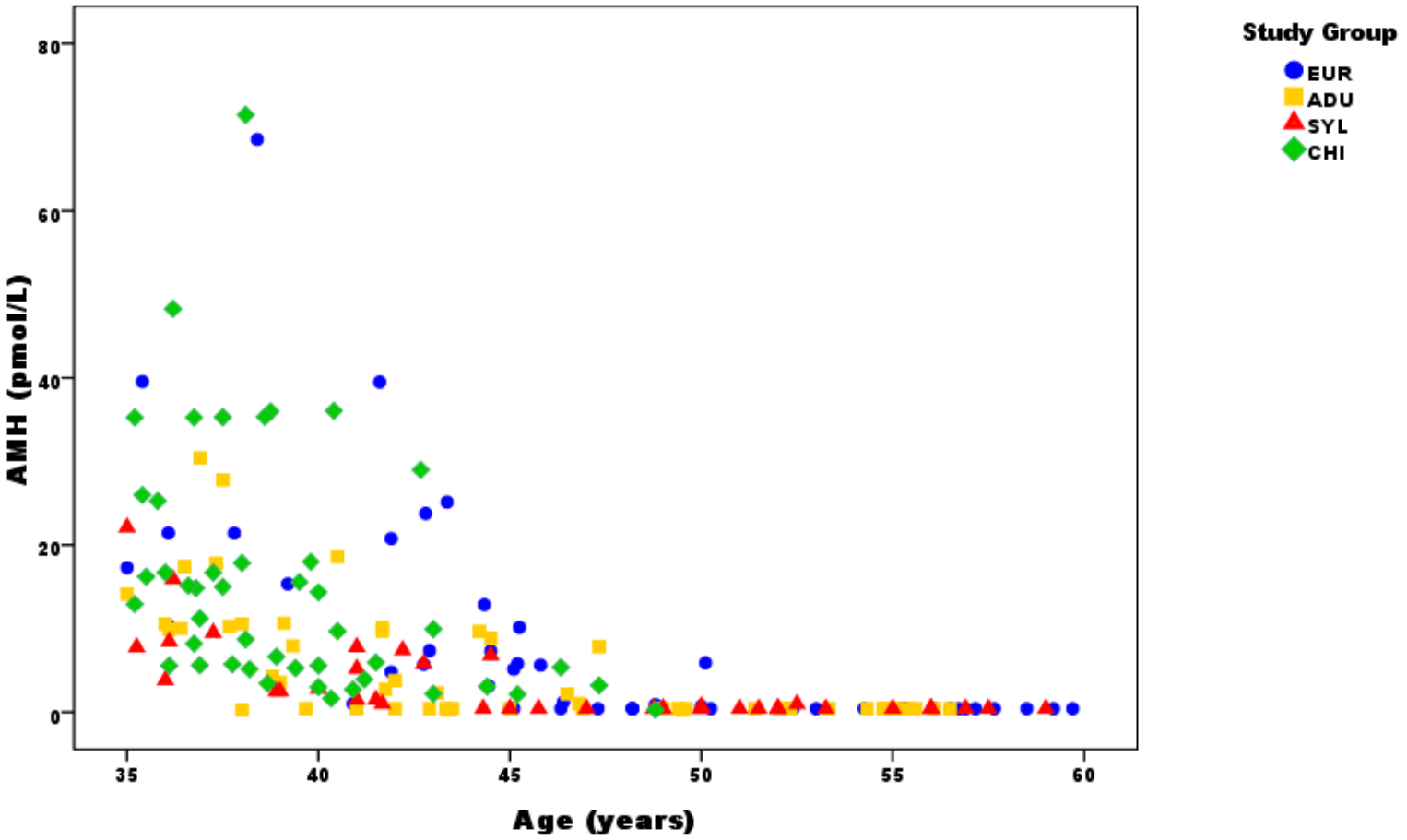


Figure 5.3.3 Correlation between FSH and age by study group

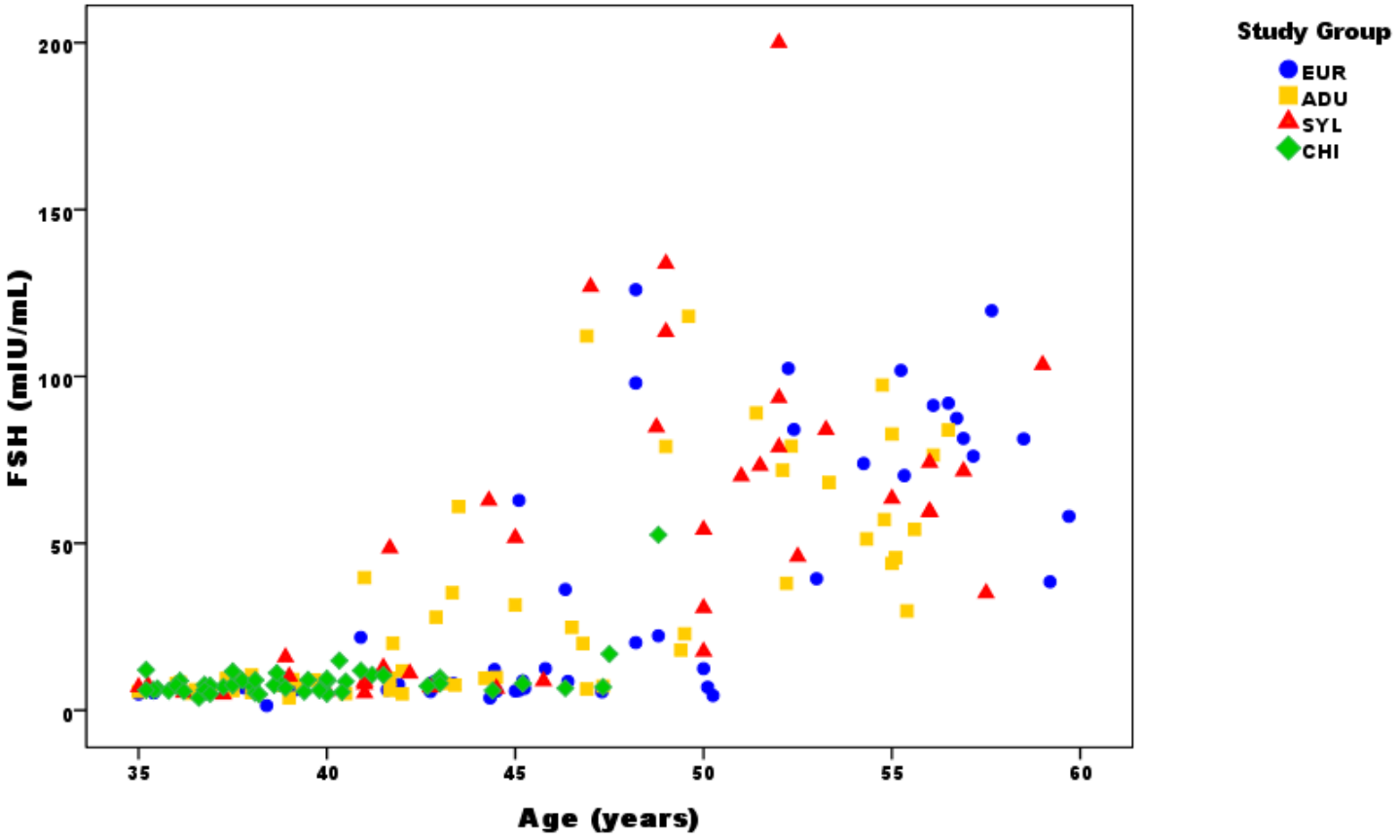


Figure 5.3.4 Correlation between inhibin B and age by study group among the premenopausal women

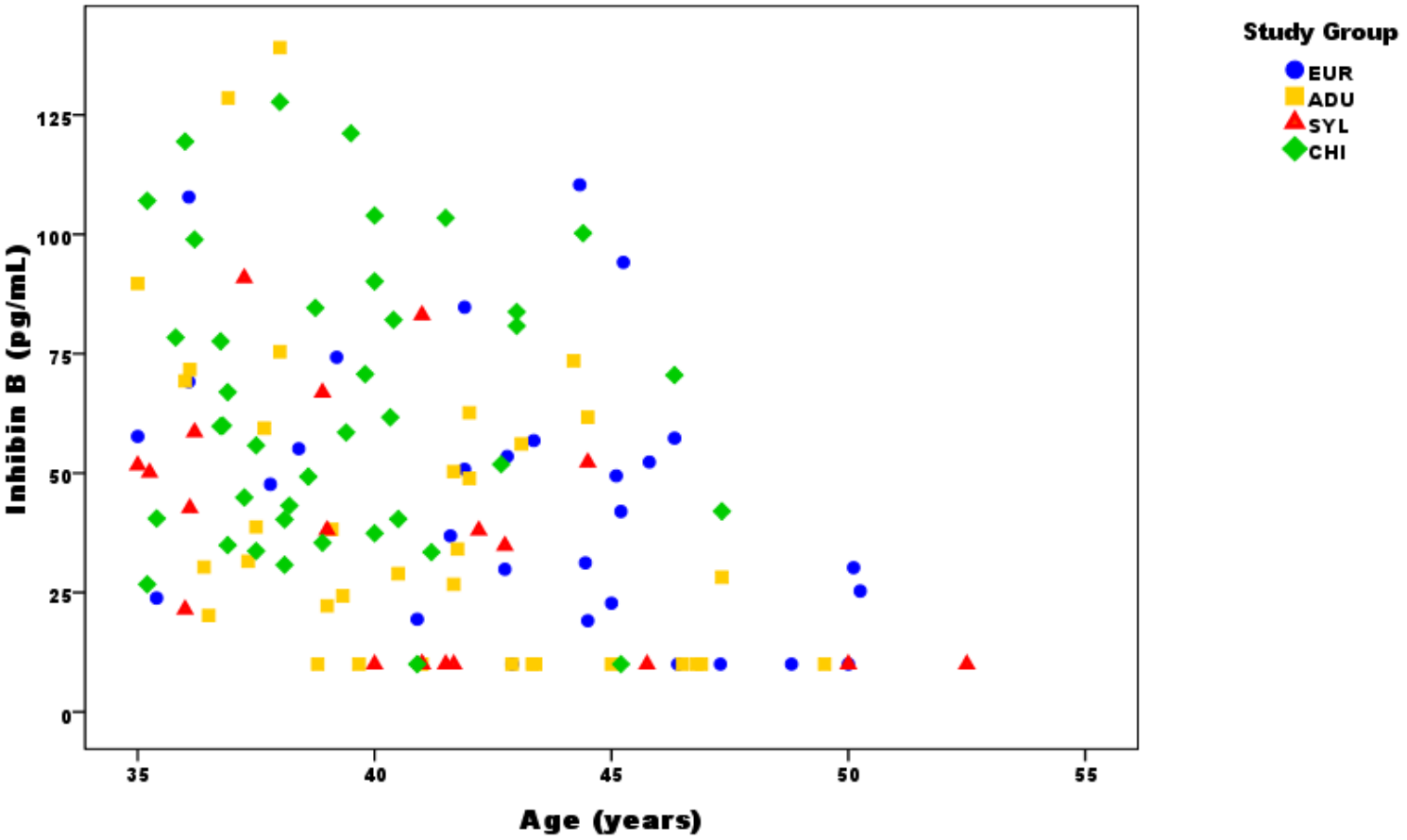


Figure 5.3.5 Correlation between AMH and age by study group among the premenopausal women

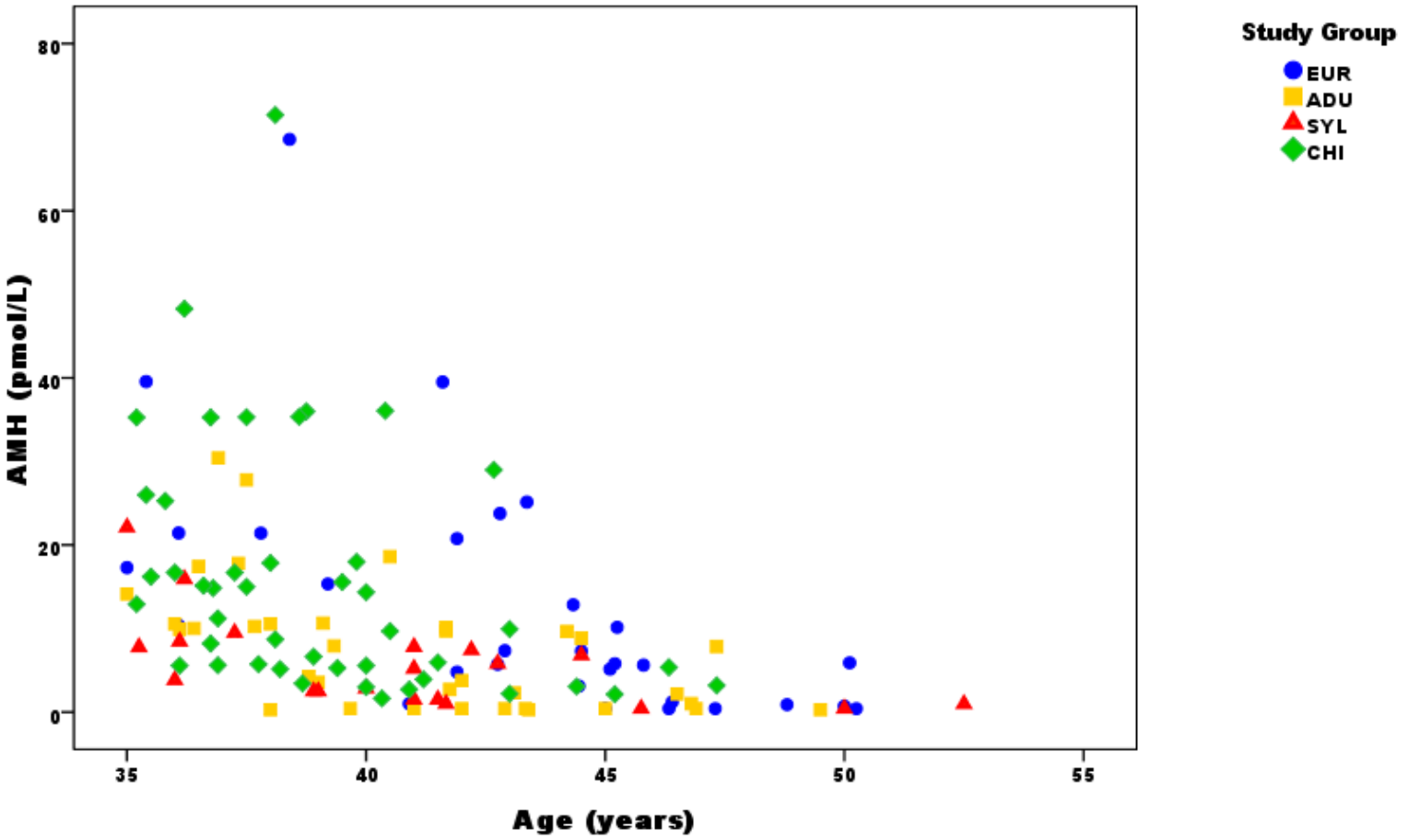
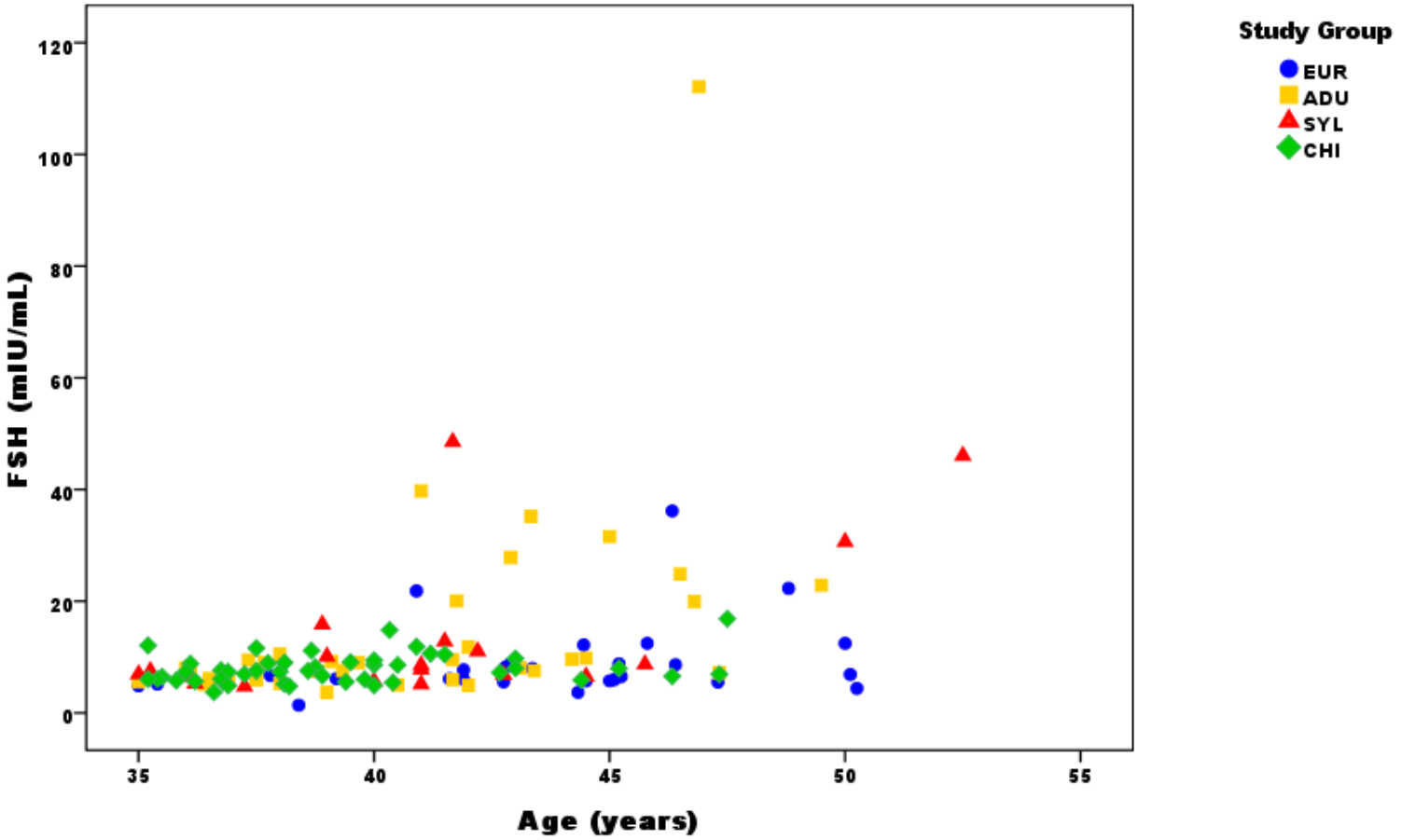


Figure 5.3.6 Correlation between FSH and age by study group among the premenopausal women



5.3.3 Age specific hormone levels between groups

Analyses of age specific hormone levels are discussed in the following part of this section. As previously described (Chapter 4), age was categorised as 35-39, 40-44, 45-49, 50-54, 55-59. Mean hormone levels are presented by age category. As the data are not normally distributed, log-transformed data and geometric means (GM) were used for analyses. For age specific hormone levels analyses, General Linear Models (univariate) were used with hormone as the dependent variable, while group and age (continuous variable) were the independent variables.

Age specific mean inhibin B levels

Results of mean inhibin B hormonal levels by age categories across the groups reveal that inhibin B levels among SYL are consistently lower in all age categories compared to EUR, CHI and ADU women except for ADU group in the 35-39 age category where SYL women had higher inhibin B (Table 5.3.8). CHI maintains higher inhibin B levels in all age categories. The mean differences are highest in the 45-49 years age category between EUR and two Bangladeshi groups (SYL and ADU) as well as between CHI and SYL and ADU. Results reveal that the EUR (24 pg/ml) has 2.5 fold and CHI (31 pg/ml) has more than 3 fold higher inhibin B levels than SYL (10 pg/ml) at the limits of

detection), while ADU (12 pg/ml) and SYL have comparable inhibin B levels in this age category (45-49). However, SYL (45-49 years) has inhibin B levels at undetectable levels earlier than ADU (50-54) and EUR (55-59), while none of the CHI has undetectable inhibin B levels (45-49), however, there were no women aged >50 years in this group. The results of univariate analysis (GLM) suggest that there is a significant difference in inhibin B levels between groups after controlling for age. Inhibin B levels are higher in CHI and EUR than SYL, but ADU and SYL have comparable inhibin B levels (Table 5.3.11).

Age specific mean AMH levels

Mean AMH levels are consistently higher in CHI and EUR women compared to ADU and SYL in the 35-39, 40-44 and 45-49 age categories. In the 55-59 year age category, AMH levels are undetectable in SYL, ADU and EUR groups (Table 5.3.9). There are no women aged >50 years in CHI group. AMH levels are undetectable earlier in the SYL (45-49) compared to ADU Bangladeshis (50-54) and EUR women (55-59). The results of univariate analysis (GLM) suggests that AMH levels are significantly (<0.001) higher in CHI and EUR compared to SYL after controlling for age, while there is no significant difference in age specific AMH levels between SYL and ADU (Table 5.3.11).

Age specific mean FSH levels

Mean FSH levels by age categories across the groups reveal that levels are almost similar with below 10 mIU/mL among women aged 35 to 40 years across the groups (Table 5.3.10). FSH levels start to rise thereafter with SYL having higher FSH levels consistently compared to ADU, CHI and EUR (Figure 5.3.9). The rise in FSH levels starts at an earlier age in SYL and ADU compared to CHI and EUR women. The increase in blood hormone levels is rapid with increasing age and higher among SYL and ADU compared to CHI and EUR. After controlling for age, univariate analysis (GLM) shows that SYL has significantly higher age specific FSH levels compared to CHI and EUR, while ADU has similar age specific FSH levels to SYL (Table 5.3.11).

Table 5.3.8 Mean inhibin B (pg/ml) levels by age category across the groups

Group	Age category														
	35-39			40-44			45-49			50-54			55-59		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	48.80	7.30	10	18.88	7.98	6	10		10	10		7	10	
ADU	16	40.63	9.91	14	26.89	6.23	10	12.25	2.35	8	10		7	10	
CHI	23	58.87	6.53	13	55.81	8.06	5	31.22	17.53						
EUR	7	51.17	9.79	11	37.11	9.11	13	24.03	7.78	7	13.96	3.23	11	11.04	1.78

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.9 Mean AMH (pmol/L) levels by age category across the groups

Group	Age category														
	35-39			40-44			45-49			50-54			55-59		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	6.92	2.44	10	2.82	1.50	6	0.42		10	0.48	0.06	7	0.42	
ADU	16	7.31	2.13	14	1.98	1.50	10	0.70	0.74	8	0.42		7	0.42	
CHI	28	15.49	2.94	13	6.38	2.98	5	2.01	0.82						
EUR	7	23.02	7.64	11	8.93	3.63	13	1.12	0.88	7	0.66	0.78	11	0.42	

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.10 Mean FSH (mIU/ml) levels by age category across the groups

Group	Age category														
	35-39			40-44			45-49			50-54			55-59		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	7.94	1.26	10	11.42	6.48	6	64.74	19.88	10	62.12	15.86	7	63.87	7.79
ADU	16	6.98	0.49	14	13.13	4.48	10	28.13	13.47	8	66.33	6.99	7	55.96	8.12
CHI	28	7.02	0.39	13	8.47	0.77	6	11.14	7.47						
EUR	7	5.09	0.84	11	7.63	1.49	13	17.54	10.91	7	26.62	15.31	11	78.61	6.51

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.11 Age specific analysis of the hormone levels using General Linear Models

Variable	Inhibin B			AMH			FSH		
	B	SE	P	B	SE	P	B	SE	P
Intercept	2.845	.149	0.000	3.697	.239	0.000	-1.135	.162	0.000
AGE	-.035	.003	0.000	-.078	.005	0.000	.055	.003	0.000
Group*									
ADU	.054	.054	0.318	.015	.087	0.862	-.085	.059	0.152
CHI	.281	.062	0.000	.343	.097	0.000	-.153	.065	0.020
EUR	.198	.055	0.000	.303	.089	0.000	-.231	.061	0.000

*Group: SYL as reference.

5.3.4 Age-specific hormone levels of the premenopausal women across groups

As previously mentioned, there is only one postmenopausal woman in the CHI group, so age specific hormone levels of the premenopausal women were further analysed. As there are very few premenopausal women aged 50-59 analyses were restricted to the age categories 35-39, 40-44 and 45-49 for this analysis.

Age specific mean inhibin B levels

Result of mean inhibin B levels by age categories among the premenopausal women suggests that SYL and ADU have lower hormonal levels compared to EUR and CHI group across the age categories (Table 5.3.12). The result also shows that CHI has the highest inhibin B levels in all the age categories. General linear model (GLM) suggests that EUR and CHI have significantly higher inhibin B levels compared to SYL after controlling for age, while there is no significant difference in inhibin B levels between ADU and SYL groups (Table 5.3.15).

Age specific mean AMH levels

Results of mean AMH levels by age categories of the premenopausal women shows that premenopausal women of SYL and ADU have consistently lower AMH levels throughout the age

categories compared to EUR and CHI (Table 5.3.13). Univariate analysis (GLM) of the AMH levels shows that after controlling for age, AMH levels of the SYL are significantly lower compared to EUR and CHI, while ADU has comparable levels of age specific AMH to SYL (Table 5.3.15).

Age specific mean FSH levels

Mean FSH levels by age categories of the premenopausal women reveal that CHI and EUR have lower FSH levels in 40-44 and >45 age categories compared to SYL and ADU (Table 5.3.14). After controlling for age, univariate analysis (GLM) shows that only EUR has significantly lower FSH levels than SYL, while there is no significant difference in age specific FSH levels between SYL and CHI, and between SYL and ADU (Table 5.3.15).

Table 5.3.12 Mean inhibin B (pg/ml) levels of the premenopausal women by age category across the groups

Group	Age category								
	35-39			40-44			45+		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	48.80	7.30	9	20.25	8.65	3	10	
ADU	16	40.63	9.91	13	29.01	6.39	6	11.89	3.03
CHI	23	58.87	6.53	13	55.81	8.06	4	31.22	19.32
EUR	7	57.17	9.79	11	37.11	9.11	12	26.14	7.44

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.13 Mean AMH (pmol/L) levels of the premenopausal women by age category across the groups

Group	Age category								
	35-39			40-44			45+		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	6.93	2.44	9	3.49	0.91	3	1.79	0.19
ADU	16	7.31	2.13	13	2.23	1.58	6	0.98	1.20
CHI	28	15.49	2.93	13	6.38	2.98	4	3.26	0.68
EUR	7	23.02	7.64	11	8.93	3.63	12	1.58	0.95

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.14 Mean FSH (mIU/ml) levels of the premenopausal women by age category across the groups

Group	Age category								
	35-39			40-44			45+		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
SYL	8	7.49	1.26	9	9.45	4.57	3	23.10	10.82
ADU	16	6.98	0.49	13	11.67	3.29	6	25.72	15.48
CHI	23	7.02	0.39	13	8.47	0.77	4	8.16	2.03
EUR	7	5.09	0.84	11	7.63	1.49	12	9.18	2.67

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.15 Age specific analysis of the hormone levels using General Linear Models of the premenopausal women

Variable	Inhibin B			AMH			FSH		
	B	**SE**	**P**	**B**	**SE**	**p**	**B**	**SE**	**P**
Intercept	2.903	0.291	0.000	4.199	0.444	0.001	-0.231	0.215	0.283
AGE	-0.036	0.007	0.000	-0.089	0.011	0.001	0.030	0.005	0.001
Group*									
ADU	0.054	0.083	0.512	-0.008	0.127	0.948	0.027	0.063	0.670
CHI	0.278	0.082	0.001	0.316	0.124	0.012	-0.068	0.060	0.259
EUR	0.222	0.087	0.012	0.408	0.133	0.003	-0.188	0.066	0.005

*Group: SYL as reference.

5.3.5 Age specific hormone levels by religion among the Bangladeshi women (ADU and SYL)

As previously mentioned, the Hindus in the SYL group vary significantly for some socio-economic characteristics such as education status, financial condition, and nutritional status (BMI) compared to the Muslims in the SYL group (see section 5.2.2). Therefore, hormone levels were further investigated to examine for any variation by religion and were compared across Bangladeshi groups. All ADU women are Muslim but the SYL includes both Muslims and Hindu; therefore, after splitting SYL group into SYL Muslim and SYL Hindus further analyses were done among three categories – ADU, SYL Hindu and SYL Muslim.

The results reveal that ADU and SYL Muslims have higher mean inhibin B levels in the 35-39 and 40-44 year age category compared to SYL Hindus (Table 5.3.16). However, univariate analysis (GLM) reveals no significant differences between SYL Hindus, SYL Muslims and ADU after controlling for age. Interestingly, after separating the Hindu women from the Muslim women in the SYL group, inhibin B levels for the SYL Muslim group across age categories become more consistent with the ADU (Figure 5.3.7). Moreover, mean inhibin B of SYL Hindus is

undetectable earlier (40-44) compared to SYL Muslims (45-49) and ADU (50-54) (Figure 5.3.7).

Mean AMH levels by religion among the Bangladeshi women are shown in table 5.3.17. In the 40-44 age category, the mean AMH levels are lowest in SYL Hindu group compared to SYL Muslim and ADU. AMH levels are undetectable after 45 years across the all groups. Variance analysis (GLM) reveals that AMH levels do not vary between groups significantly across the age categories. After separating Hindus from Muslims, the difference in AMH levels between SYL Muslim and ADU is reduced (Figure 5.3.8).

Results of FSH levels by religion among the Bangladeshi women suggest that, although mean FSH levels are similar in the 35-39 age category across groups (Table 5.3.18), SYL Hindus have higher FSH levels in other age categories. Moreover, after separating Hindus, FSH levels of the SYL Muslims become closer to the ADU (Figure 5.3.9). Univariate analysis (GLM) reveals no significant difference in FSH levels between groups. Therefore, it can be concluded that the higher levels of FSH in Hindu SYL confound the difference in FSH levels between SYL and ADU.

Although the results suggest the lower inhibin B and AMH levels and higher FSH levels of the SYL Hindu women influence the

differences in age specific hormone levels between SYL and ADU, the numbers of women in each age category are too small to understand the actual situation. Therefore, it needs to be further investigated with a larger sample size in the future.

Table 5.3.16 Mean inhibin B (pg/ml) levels of ADU and SYL by religion and age category

Age category	Group								
	ADU			SYL Muslim			SYL Hindu		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
35-39	16	40.63	9.91	5	56.95	9.07	2	35.46	18.55
40-44	14	26.89	6.23	8	22.13	9.44	2	10.00	.00
45-49	10	12.25	2.35	3	10.00	.00	3	10.00	.00
50-54	8	10		8	10.00	.00	2	10.00	.00
55-59	7	10		1	10.00	.00	5	10.00	.00
Total	55	20.07	4.07	25	18.26	5.09	14	11.98	3.50

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.17 Mean AMH (pmol/L) levels of ADU and SYL by religion and age category

Age category	Group								
	ADU			SYL Muslim			SYL Hindu		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
35-39	16	7.30	9.16	5	6.34	3.59	2	7.83	6.04
40-44	14	1.97	5.69	8	3.48	0.99	2	1.22	.25
45-49	10	0.69	2.61	3	0.42	.00	3	0.42	.00
50-54	8	.42	.00	8	0.45	.03	2	0.64	.29
55-59	7	.42	.00	1	0.42	.00	5	.42	.00
Total	55	1.57	.95	25	1.45	.99	14	0.79	1.10

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Table 5.3.18 Mean FSH (mIU/mL) levels of ADU and SYL by religion and age category

Age category	Group								
	ADU			SYL Muslim			SYL Hindu		
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM
35-39	16	6.98	0.49	5	8.33	1.91	2	6.18	.98
40-44	14	13.13	4.48	8	9.87	6.92	2	20.38	19.98
45-49	10	28.13	13.47	3	45.48	34.58	3	92.19	24.70
50-54	8	66.33	6.99	8	62.59	19.69	2	60.26	16.41
55-59	7	55.96	8.12	1	63.45		5	72.08	8.06
Total	55	19.10	4.33	25	22.30	9.72	14	43.54	10.40

Note: Mean indicates Geometric Mean, SEM indicates Standard Error of Means

Figure 5.3.7 Mean inhibin B hormone levels by religion and age category among ADU and SYL

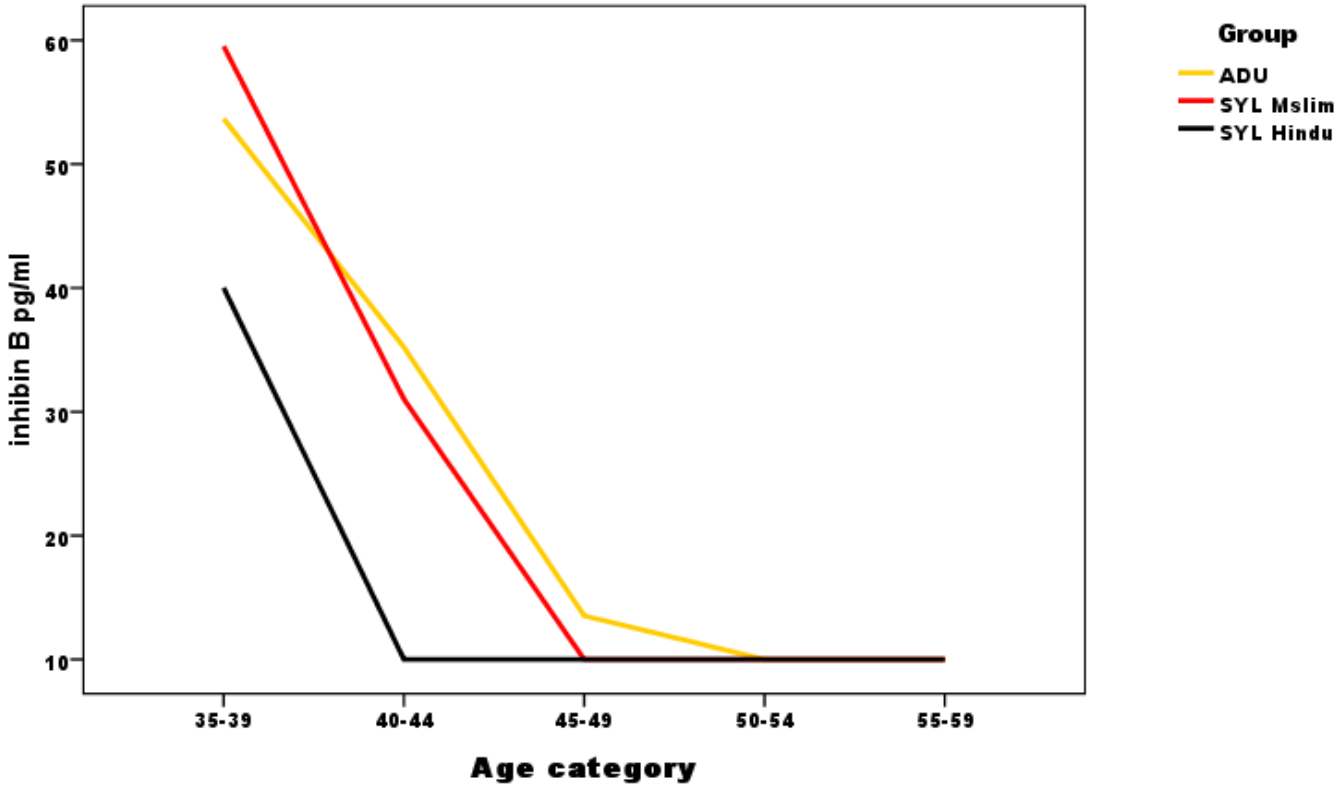


Figure 5.3.8 Mean AMH hormone levels by religion and age category among ADU and SYL

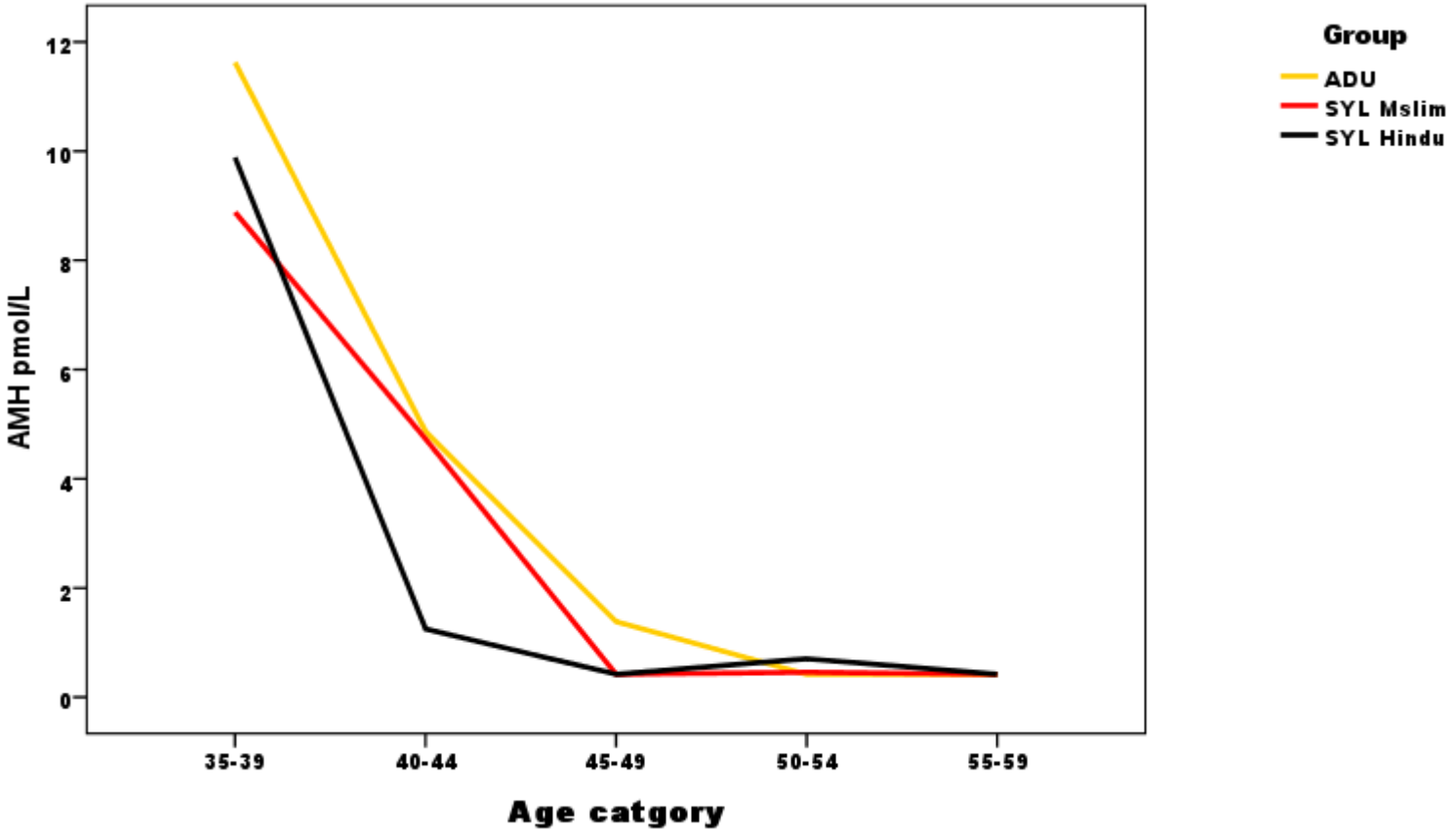
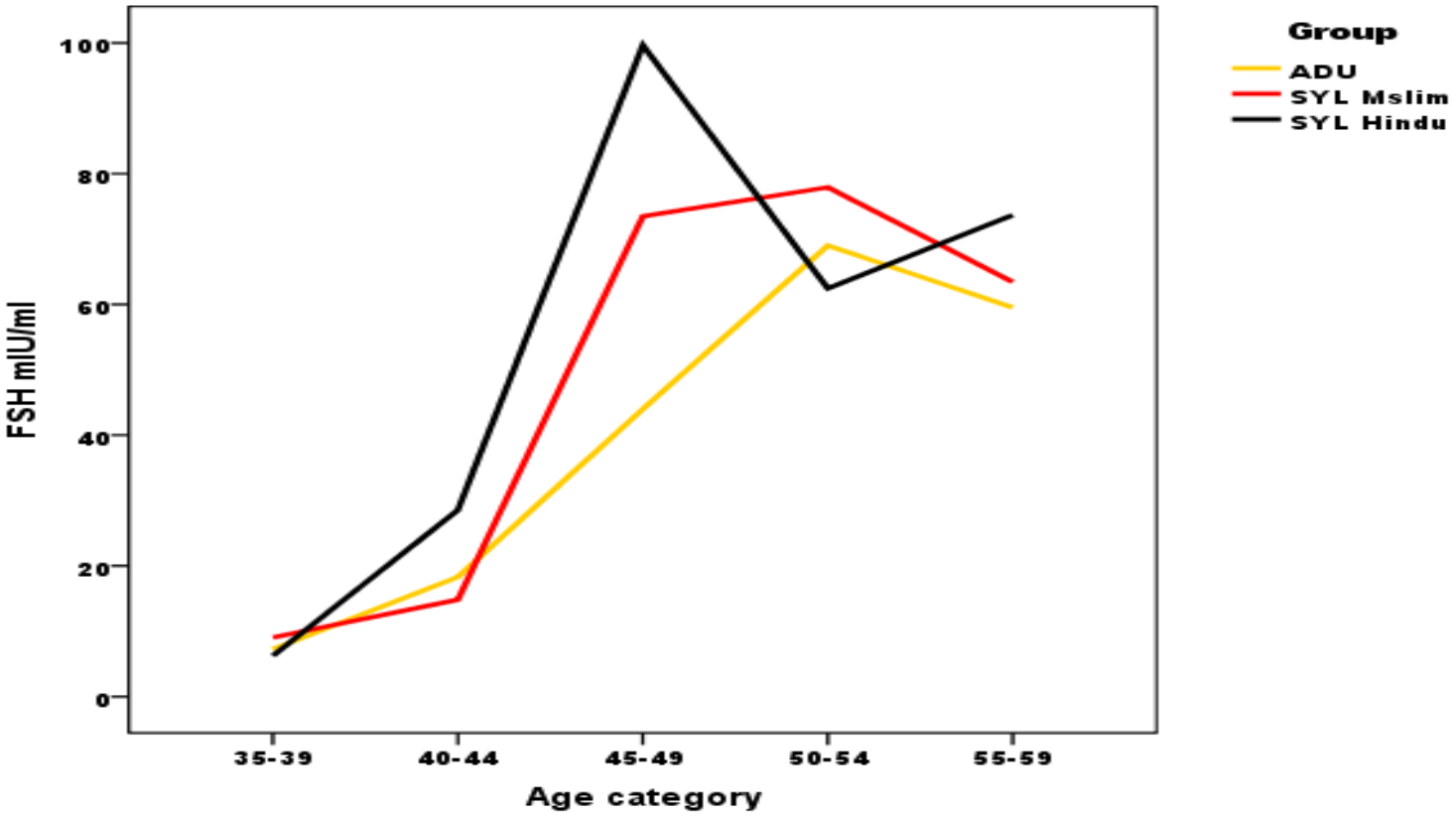


Figure 5.3.9 Mean FSH hormone levels by religion and age category among ADU and SYL



5.3.6 Hormone analysis by age at migration among CHI

In order to examine the impact of age at migration of the child migrant (CHI) on the hormonal levels, further analysis of the hormone data of CHI group were done by their age at migration, therefore, CHI women are divided into two groups as migration before 8 years and after 8 years. The results of the three hormone levels of CHI group by age at migration category are depicted in table 5.3.19 and figure 5.3.10-12. The result reveals that the former (43.77 ± 7.28) has lower inhibin B levels compared to the latter (61.04 ± 6.41), conversely, for AMH levels, before 8 years group (9.41 ± 3.84) has higher than after 8 years group (8.58±1.99). On the other hand, FSH levels are similar between two groups (8.46 ± 0.57 vs 7.73 ± 0.548). However, the hormones levels are not significantly different between two groups.

Table 5.3.19 Mean hormone levels by age at migration of CHI

	Age at migration	N	Mean	SEM	Statistics*
Inhibin B	< 8 years	16	43.77	7.28	$t_{26.93}$= -1.472; p=0.153 (ns)
	> 8 years	20	61.04	6.41	
AMH	<8 years	18	9.41	3.84	$t_{26.939}$=0.251; p=0.804 (ns)
	>8 years	23	8.58	1.99	
FSH	<8 years	18	8.46	0.57	$t_{23.857}$=0.608; p=0.549 (ns)
	>8 years	24	7.73	.548	

Note: < 8 years = migration before age of 8 year
>8 years= migration after age of 8 year
Mean indicates Geometric Mean,
SEM indicates Standard Error of Means

Figure 5.3.10 Distribution of inhibin of the CHI B by age category according to age at migration

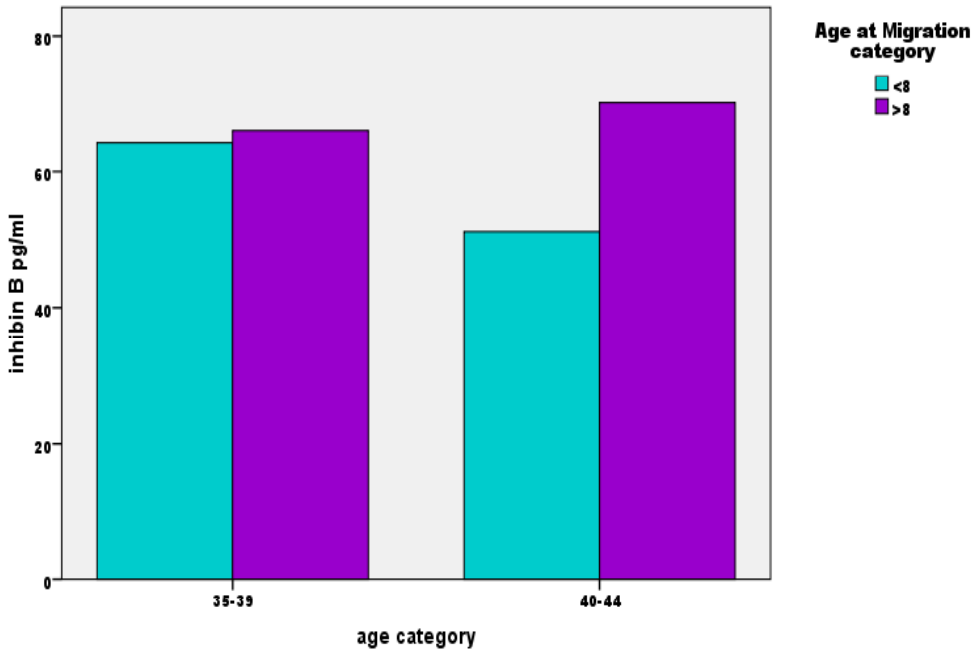


Figure 5.3.11 Distribution of AMH of the CHI by age category according to age at migration

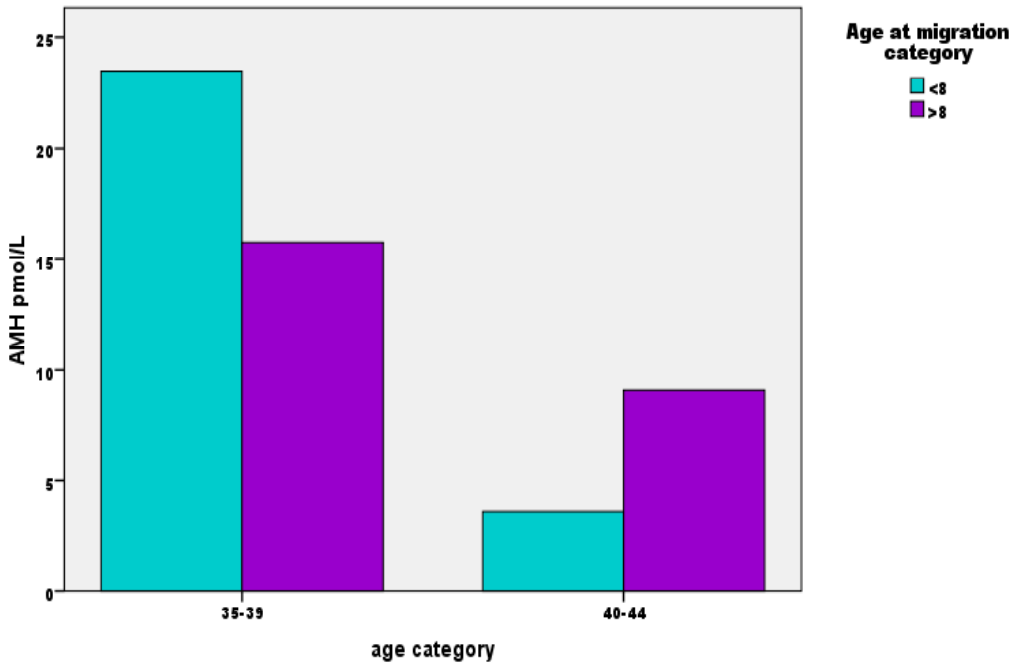
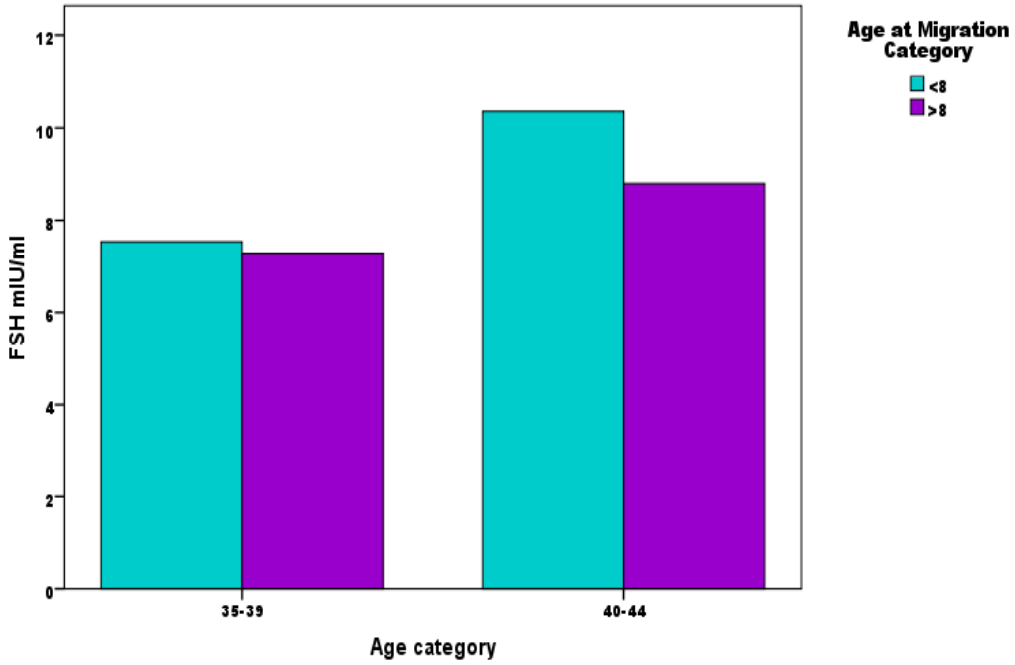


Figure 5.3.12 Distribution of FSH of the CHI by age category according to age at migration



5.4 Hypothesis testing:

In this section, results of the blood tests for the three hormones were analysed to test the three hypotheses that have been set for this study. These hypotheses are set in the context of the "developmental hypothesis" for reproductive function described by Ellison (1996).

Hypothesis 1

As early life developmental conditions impact on later life reproductive hormonal levels, there is inter-population variation in reproductive hormone levels as well as ovarian reserve. Therefore, women who grow up in an adverse environment will have lower levels of age-related ovarian reserve compared to women who grow up in a better environment.

Prediction: Bangladeshi women who grew up in Bangladesh will have a lower ovarian reserve compared to women of European descent.

Multiple linear regression analysis (MLR) was performed with the dependent variables as log transformed hormonal data and independent variables being age, BMI, height, menopausal status (categorised as pre and post menopause) and groups . There was no multicollinearity of the independent variables.

MLR suggests that after controlling for age, BMI, height and menopausal status, inhibin B levels are significantly different between groups (R^2 = 0.514, p<0.001; Adj. R^2 = 0.493; Table 5.4.1). Results suggest that age specific inhibin B levels are significantly different between SYL and EUR though there is no significant difference between SYL and ADU. Further MLR analysis with stepwise method suggests that only Age and EUR group have a significant effect on inhibin B levels.

Results using MLR show significant differences in AMH levels between groups (R^2 = 0.633, p<0.001, Adj. R^2 = 0.617). AMH levels are significantly higher in EUR group compared to SYL after controlling for age, BMI, height and menopausal status (Table 5.4.2). However, there is no significant difference in AMH levels between SYL and ADU. A stepwise MLR model reveals that age, EUR and menopausal status have a significant effect on AMH levels.

Results of MLR with predictors age, BMI, height, menopausal status and groups suggest that individual contributions of the independent variables to differences in FSH levels between groups are significant (R^2 = 0.514, p<0.001, Adj. R^2 = 0.493) (Table 5.4.3). The result suggests that after controlling for age, BMI, height and menopausal status, FSH levels are still significantly different between SYL and EUR but not between SYL and ADU.

Further analysis with stepwise MLR reveals that menopausal status, age and EUR can significantly predict FSH level.

Figures 5.4.1-3 indicate trends of inhibin B, AMH and FSH hormonal levels respectively across the age categories.

Table 5.4.1 Multiple Linear Regression Model for Inhibin B Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		3.466	.577		6.002	.000
Age		-.031	.005	-.594	-5.965	.000
Anthropometric	**BMI**	-.008	.005	-.100	-1.634	.105
	Height	-.003	.004	-.068	-.887	.337
Reproductive†	**Menopausal Status**	-.095	.073	-.131	-1.303	.195
Group‡	**ADU**	.073	.055	.098	1.321	.189
	EUR	.220	.068	.289	3.239	.002

Dependent inhibin B (Log10 inhibin B)
$F_{6,138}$=24.335, p<0.001; R^2 = 0.514, Adj. R^2 = 0.493
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Table 5.4.2 Multiple Linear Regression Model for AMH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		2.602	0.952		2.733	0.007
Age		-0.056	0.009	-0.563	-6.500	0.000
Anthropometric	**BMI**	-0.002	0.008	-0.013	-0.243	0.808
	Height	0.004	0.006	0.051	0.760	0.448
Reproductive†	**Menopausal Status**	-0.354	0.121	-0.256	-2.930	0.004
Group‡	**ADU**	-0.007	0.091	-0.005	-0.081	0.936
	EUR	0.217	0.097	0.150	2.225	0.028

Dependent inhibin B (Log10 inhibin B)
$F_{6,138}$=39.602, p<0.001*; R^2 = 0.633, Adj. R^2 = 0.617
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Table 5.4.3 Multiple Linear Regression Model for FSH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		-0.669	0.595		-1.125	0.263
Age		0.026	0.005	0.360	4.193	0.000
Anthropometric	**BMI**	0.000	0.004	-0.004	-0.064	0.949
	Height	0.004	0.005	0.031	0.689	0.492
Reproductive†	**Menopausal Status**	0.546	0.075	0.535	7.239	0.000
Group‡	**ADU**	-0.059	0.057	-0.057	-1.051	0.295
	EUR	-0.125	0.058	-0.117	-2.140	0.034

Dependent inhibin B (Log10 inhibin B)
$F_{6,138}$=24.374, p<0.001*; R^2 = 0.514, Adj. R^2 = 0.493
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Figure 5.4.1 Mean inhibin B by age category across the study groups (SYL, ADU & EUR)

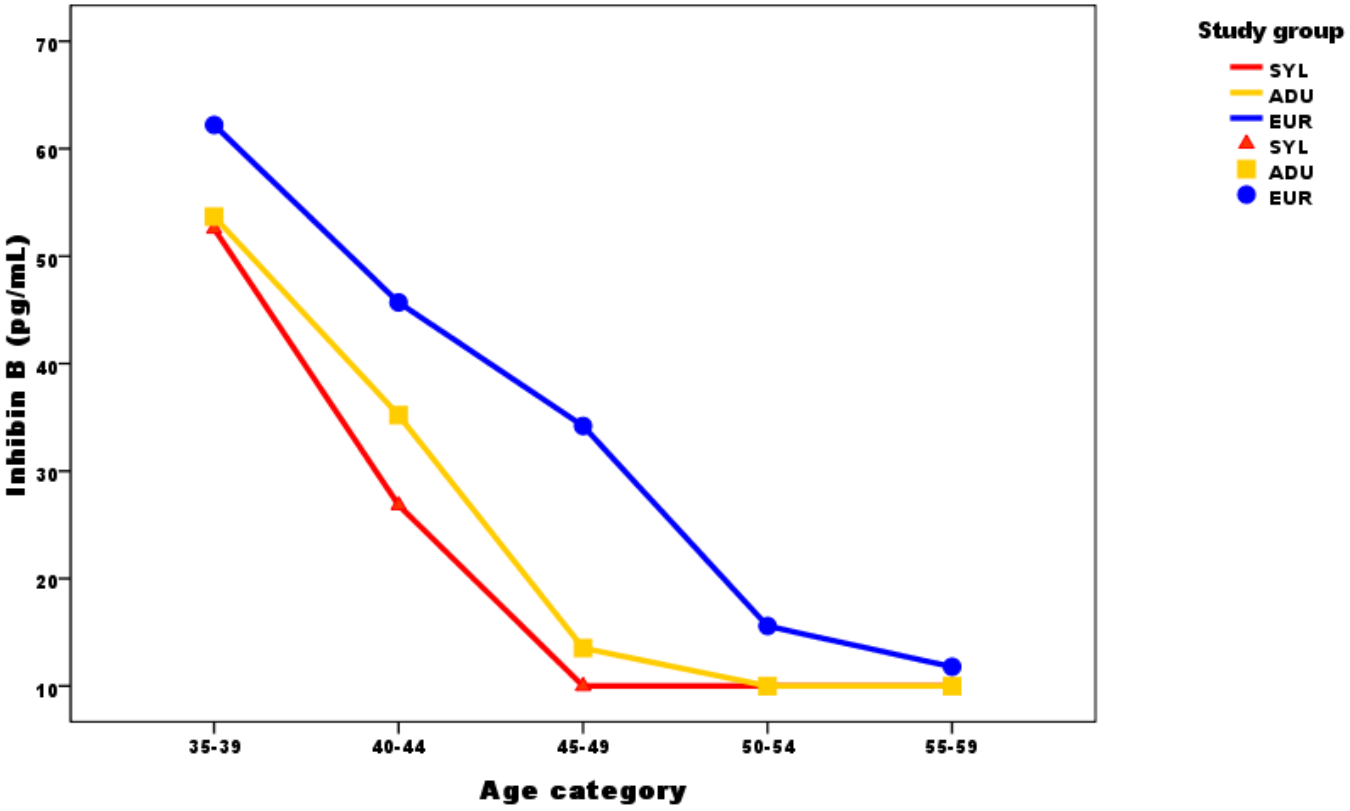


*Figure indicates trend only.

Figure 5.4.2 Mean AMH by age category across the study groups (SYL, ADU & EUR)

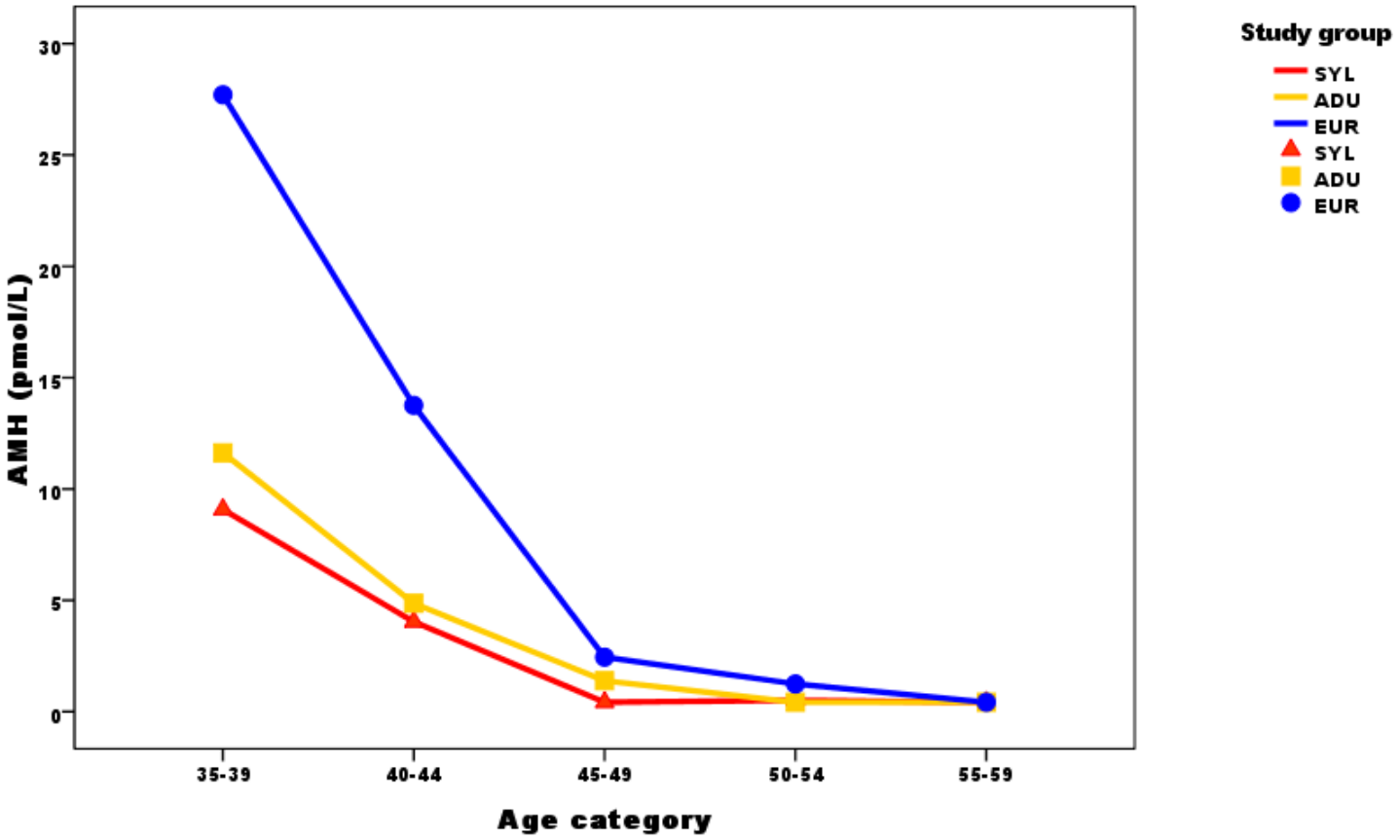


*Figure indicates trend only

Figure 5.4.3 Mean FSH by age category across the study groups (SYL, ADU & EUR)

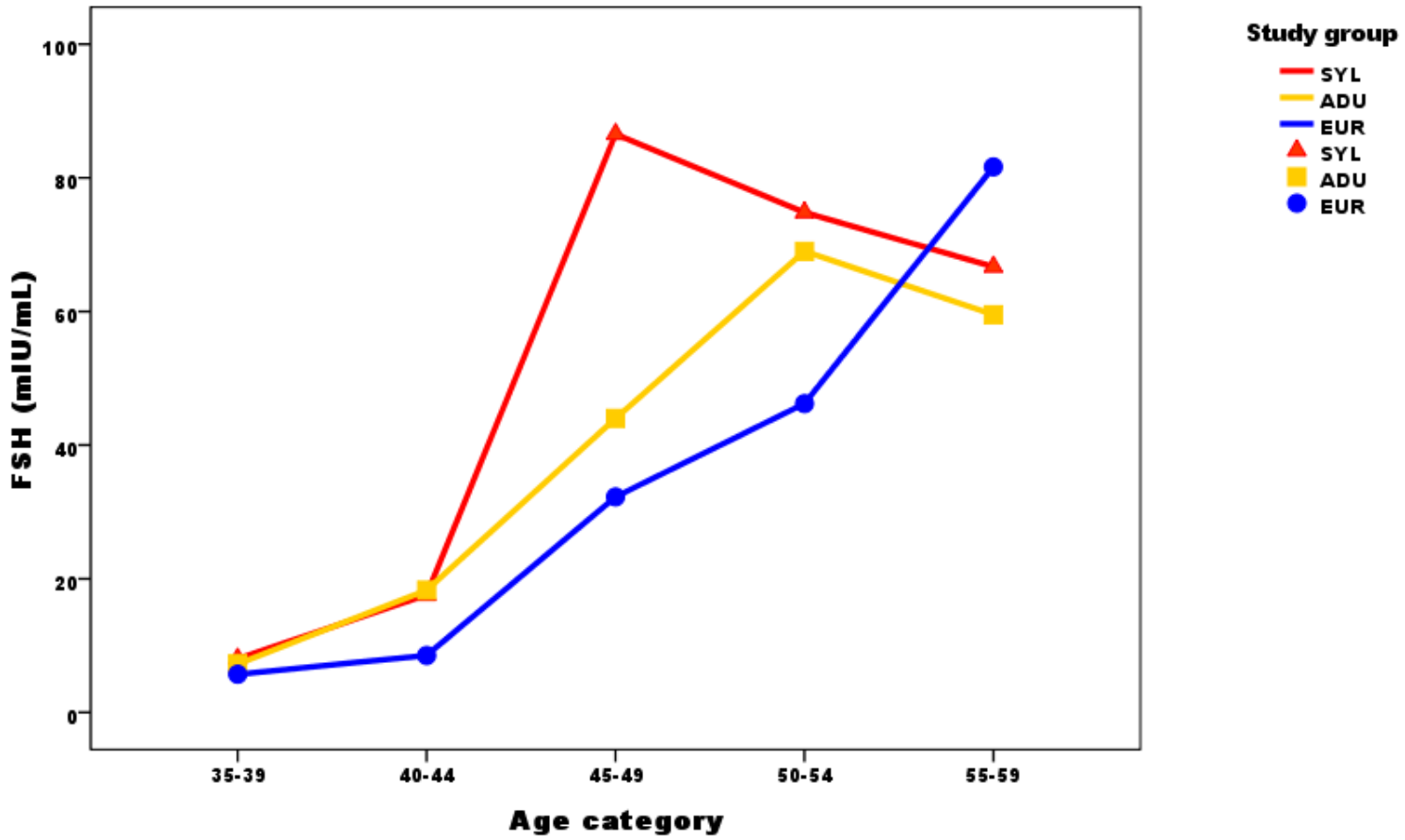


*Figure indicates trend only

Hypothesis 2:

Growing up in an adverse environment and migration to a better environment during adult life does not affect ovarian reserve.

Prediction: Women who grew up in Bangladesh and migrated to the UK as adults will have an ovarian reserve that is comparable to sedentees.

Multiple linear regression analysis suggests that all three hormones (inhibin B, AMH and FSH) levels are not significantly different between SYL and ADU after controlling for age, BMI, height and menopausal status (Table 5.4.7). The hormonal levels trends are indicated in figure 5.4.4-6. Further analyses with stepwise MLR analysis reveals that age has significant effect on inhibin B, AMH and FSH, in addition, menopausal status has a significant effect on AMH and FSH. The multicollinearity test suggests there was no multicollinearity of independent variables.

Table 5.4.4 Multiple Linear Regression Model for Inhibin B Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		3.344	0.736		4.547	0.000
Age		-0.034	0.006	-0.667	-5.339	0.000
Anthropometric	**BMI**	-0.014	0.007	-0.160	-2.070	0.041
	Height	-0.001	0.004	-0.016	-0.203	0.839
Reproductive†	**Menopausal Status**	-0.045	0.089	-0.064	-0.509	0.612
Group‡	**ADU**	0.082	0.055	0.115	1.493	0.139

Dependent inhibin B (Log10 inhibin B)
$F_{5,90}$=18.993, p<0.001; R^2 = 0.513, Adj. R^2 = 0.486
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Table 5.4.5 Multiple Linear Regression Model for AMH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		1.379	1.181		1.167	0.246
Age		-0.049	0.010	-0.525	-4.753	0.000
Anthropometric	**BMI**	-0.008	0.011	-0.049	0.722	0.472
	Height	0.011	0.007	0.114	1.644	0.104
Reproductive†	**Menopausal Status**	-0.336	0.114	-0.260	-2.341	0.021
Group‡	**ADU**	0.002	0.089	0.018	0.018	0.985

Dependent AMH (Log10 AMH)
$F_{5,90}$=29.256, p<0.001; R^2 = 0.619, Adj. R^2 = 0.598
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Table 5.4.6 Multiple Linear Regression Model for FSH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		0.083	0.782		0.106	0.916
Age		0.033	0.007	0.466	4.818	0.000
Anthropometric	**BMI**	0.001	0.007	0.009	0.152	0.880
	Height	-0.005	0.005	-0.069	-1.143	0.256
Reproductive†	**Menopausal Status**	0.377	0.095	0.386	3.963	0.000
Group‡	**ADU**	-0.056	0.059	-0.057	-0.959	-0.959

Dependent FSH (Log10 FSH)
$F_{5,90}$=43.757, p<0.001; R^2 = 0.709, Adj. R^2 = 0.692
‡Group: SYL as reference
†Menopausal status: categorised as pre and post- menopausal; Premenopausal as reference.

Figure 5.4.4 Mean inhibin B by age category across the study groups (SYL & ADU)

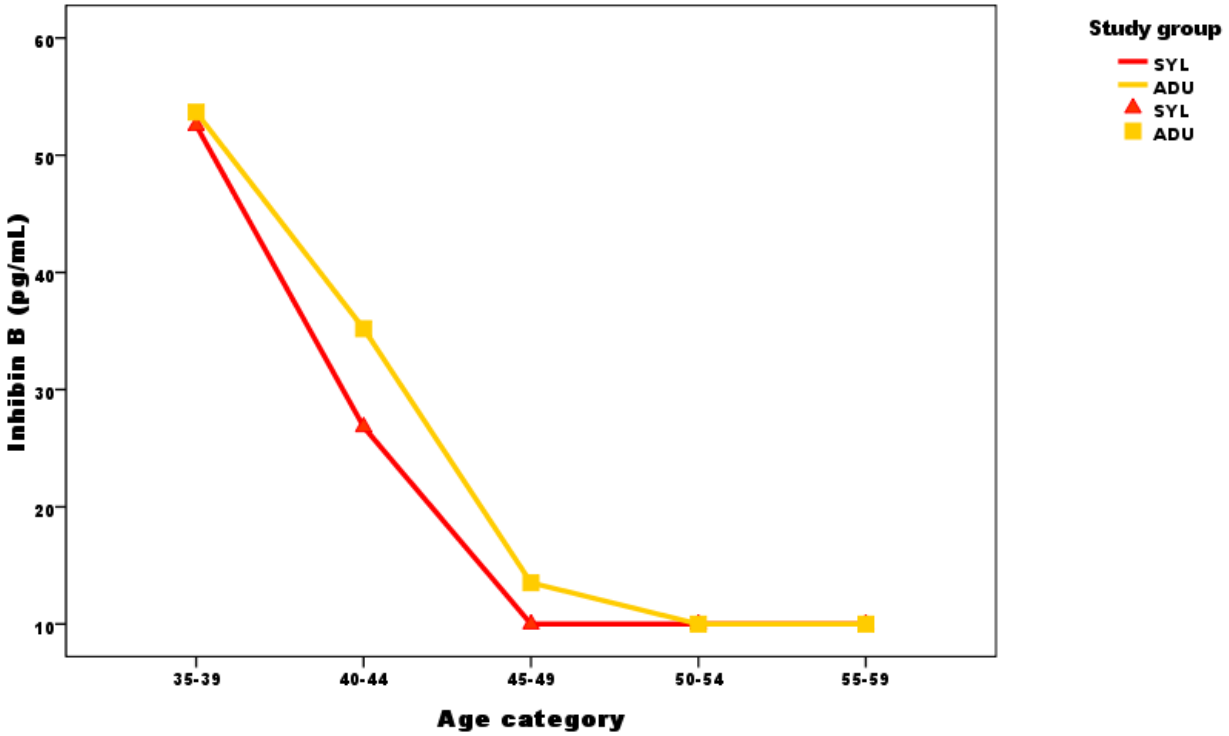


*Figure indicates trend only

Figure 5.4.5 Mean AMH by age category across the study groups (SYL & ADU)

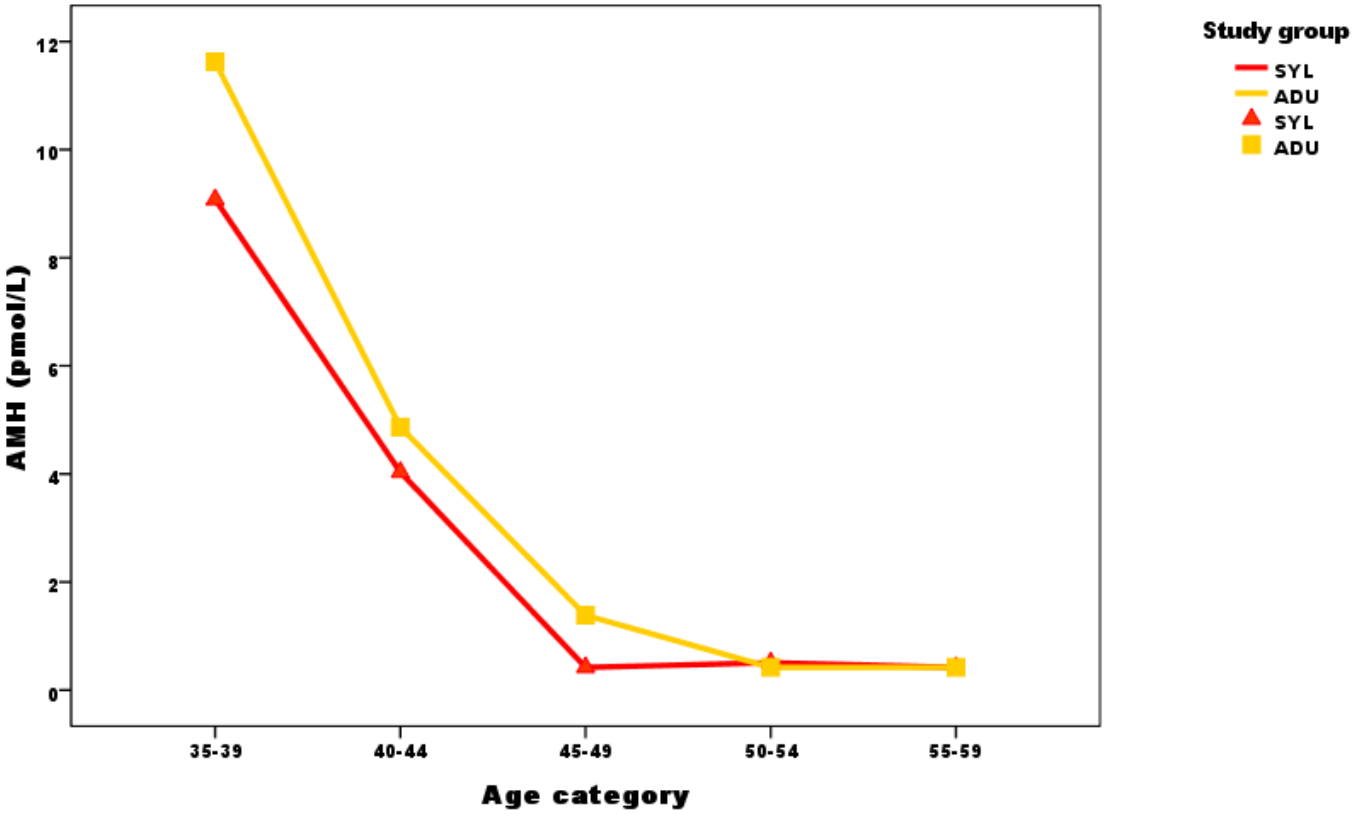


*Figure indicates trend only

Figure 5.4.6 Mean FSH by age category across the study groups (SYL & ADU)

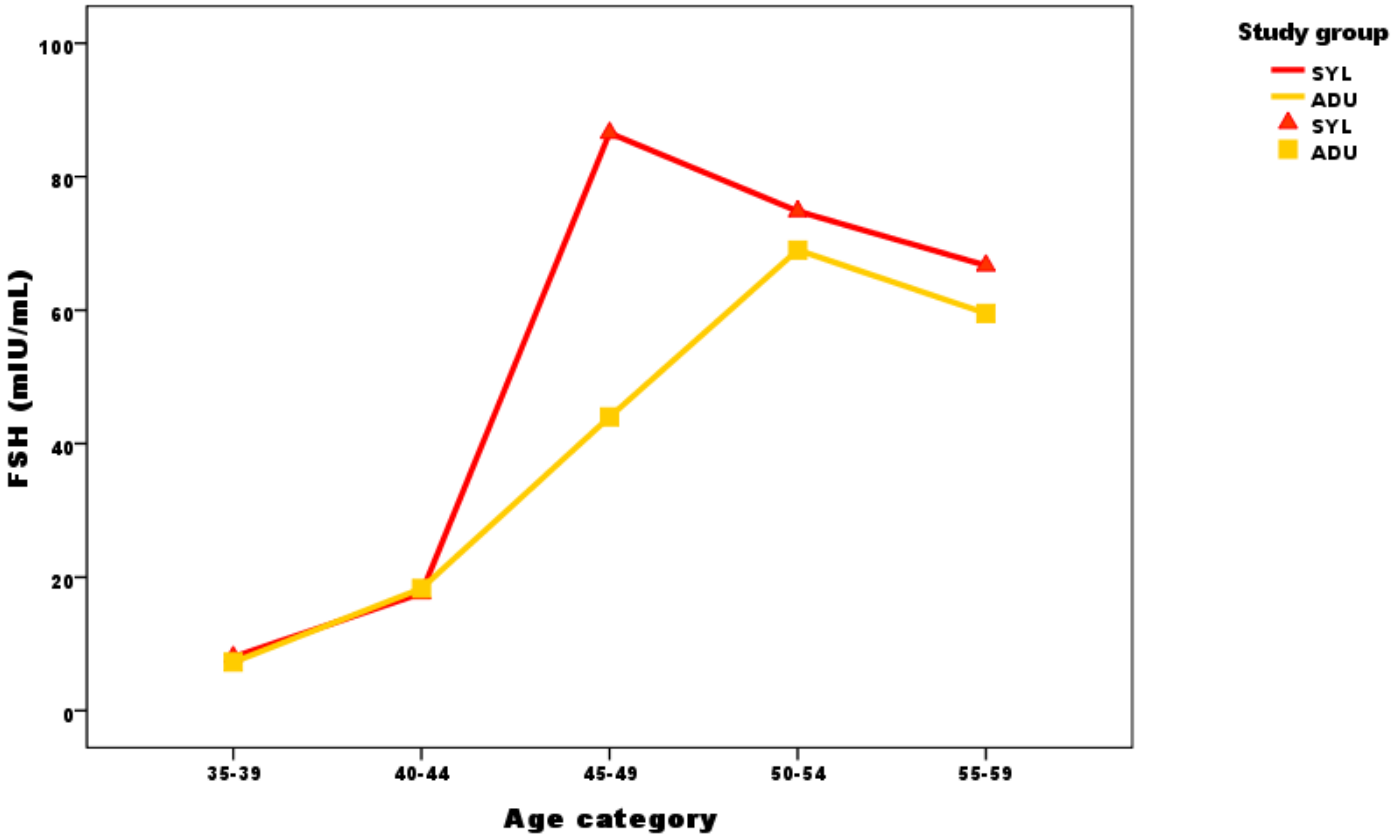


*Figure indicates trend only

***Hypothesis 3**:*

The childhood environment has an impact on age related ovarian reserve in later life. Therefore, migration to a better environment during childhood will result in a higher ovarian reserve compared to women still in the community of origin and adult migrants.

Prediction: Bangladeshi women who migrated to the UK during childhood will have a higher ovarian reserve compared to sedentees and adult migrant Bangladeshi women who spent their childhood in Bangladesh. In other words, Bangladeshi migrants who moved to the UK as children have a later decline in ovarian reserve compared to women who grew up in Bangladesh.

As the CHI group does not have any woman older than 49 and have only one post menopausal women, the analyses were performed among the premenopausal women aged between 35 and 49 years. This resulted in 18 SYL, 34 ADU, 46 CHI and 27 EUR in the dataset used for the analysis. Entering predictors simultaneously in the MLR reveals that, after controlling for age, BMI and height, inhibin B levels are significantly ($F_{6,\ 112}$=6.897, $p<0.001$; R^2= 0.270, Adj. R^2 =0.231) different between the groups (Table 5.4.7). After controlling for age, BMI and height, inhibin B levels are found to be significantly lower in SYL

compared to CHI and EUR, but comparable to ADU (Figure 5.4.7). For further analysis stepwise MLR analysis was done and reveals that only age and groups (CHI and EUR) have significant effect on inhibin B levels.

For AMH, MLR shows significant differences between groups ($F_{6,117}$=12.663, p<0.001; R^2 = 0.394, Adj. R^2 = 0.363) (Table 5.4.8). AMH levels of CHI and EUR are significantly higher compared to SYL after controlling for age, BMI and height (Figure 5.4.8), while AMH levels of ADU are not different from AMH levels of SYL. Further MLR analysis with stepwise method reveals that age and groups (EUR and CHI) have significant effects on AMH levels.

MLR controlling for age, BMI and height, show significant differences between groups for FSH ($F_{6,118}$ =5.606, p<0.00; R^2 = 0.222, Adj. R^2 = 0.182; Table 5.4.9). Average FSH levels are not significantly higher in SYL compared to ADU, CHI and EUR (Table 5.4.9).

Table 5.4.7 Multiple Linear Regression Model for Inhibin B Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		3.715	.752		4.939	.000
Age		-.033	.008	-.341	-3.940	.000
Anthropometric	**BMI**	-.004	.004	-.091	-.981	.329
	Height	-.011	.007	-.143	-1.678	.096
Group‡	**ADU**	.069	.088	.092	.783	.435
	CHI	.292	.085	.408	3.429	.001
	EUR	.226	.101	.279	2.230	.028

Dependent inhibin B (Log10 inhibin B)
$F_{6, 112}$=6.897, p<0.001; R^2= 0.270, Adj. R^2 =0.231
‡Group: SYL as reference

Table 5.4.8 Multiple Linear Regression Model for AMH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		4.296	1.129		3.806	.000
Age		-.091	.013	-.564	-7.252	.000
Anthropometric	**BMI**	.000	.007	-.011	-.139	.890
	Height	.003	.010	.024	.315	.753
Group‡	**ADU**	.021	.135	.016	.153	.879
	CHI	.333	.129	.279	2.573	.011
	EUR	.452	.156	.325	2.905	.004

Dependent AMH (Log10 AMH)
$F_{6,117}$=12.663, p<0.001; R^2 = 0.394, Adj. R^2 = 0.363
‡Group: SYL as reference

Table 5.4.9 Multiple Linear Regression Model for FSH Index

Independent Variables	Category	Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	t-value	p-value
(Constant)		-.158	.544		-.290	.772
Age		.028	.006	.416	4.800	.000
Anthropometric	**BMI**	.000	.003	-.025	-.273	.785
	Height	.004	.005	.063	.741	.460
Group‡	**ADU**	.052	.065	.096	.805	.422
	CHI	-.043	.062	-.085	-.697	.487
	EUR	-.115	.075	-.193	-1.526	.130

Dependent FSH (Log10 FSH)
$F_{6,118}$ =5.606, p<0.00; R^2 = 0.222, Adj. R^2 = 0.182
‡Group: SYL as reference

Figure 5.4.7 Mean inhibin B by age category across the study groups (SYL, ADU, CHI and EUR)

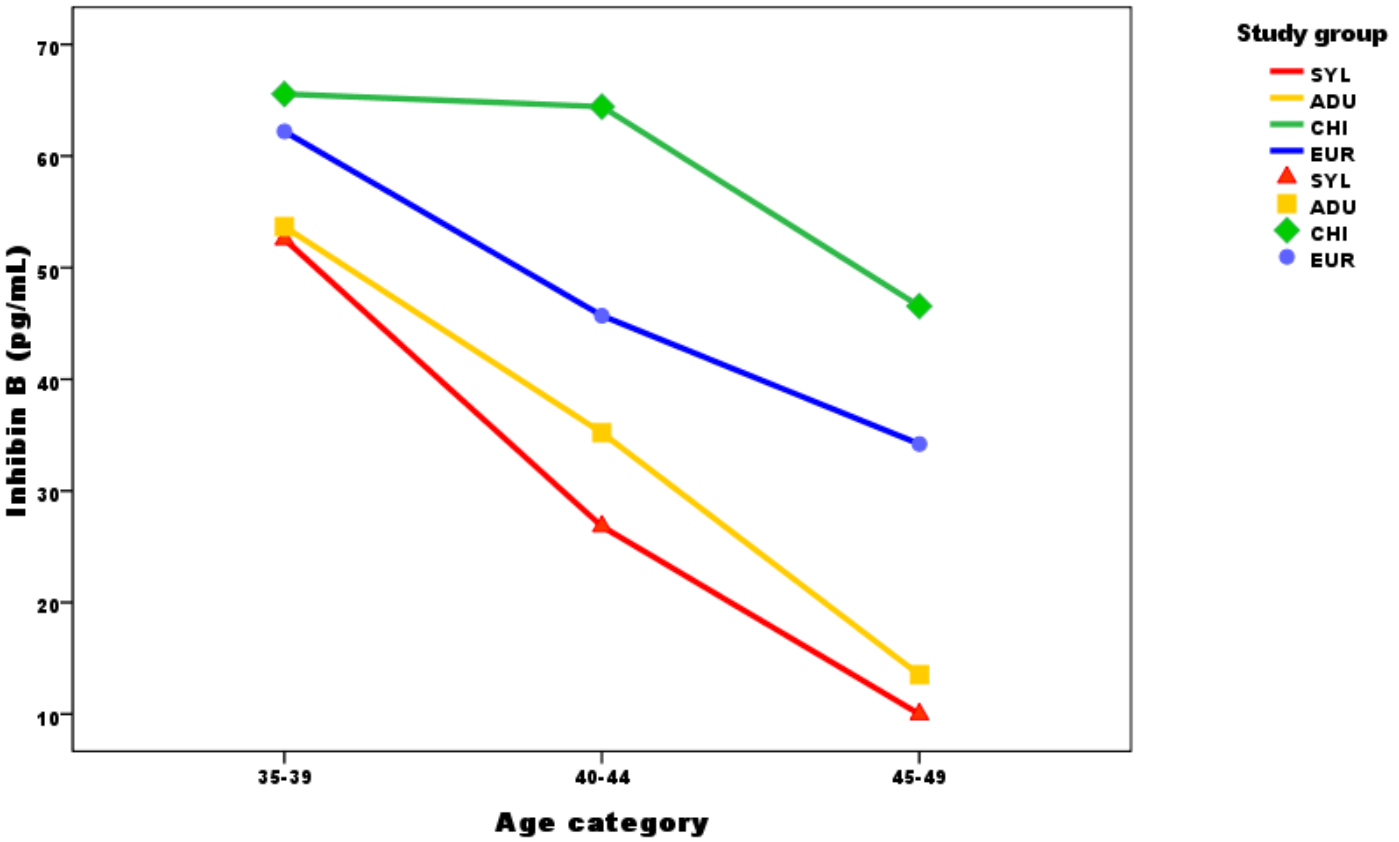


*Figure indicates trend only

Figure 5.4.8 Mean AMH by age category across the study groups (SYL, ADU, CHI and EUR)

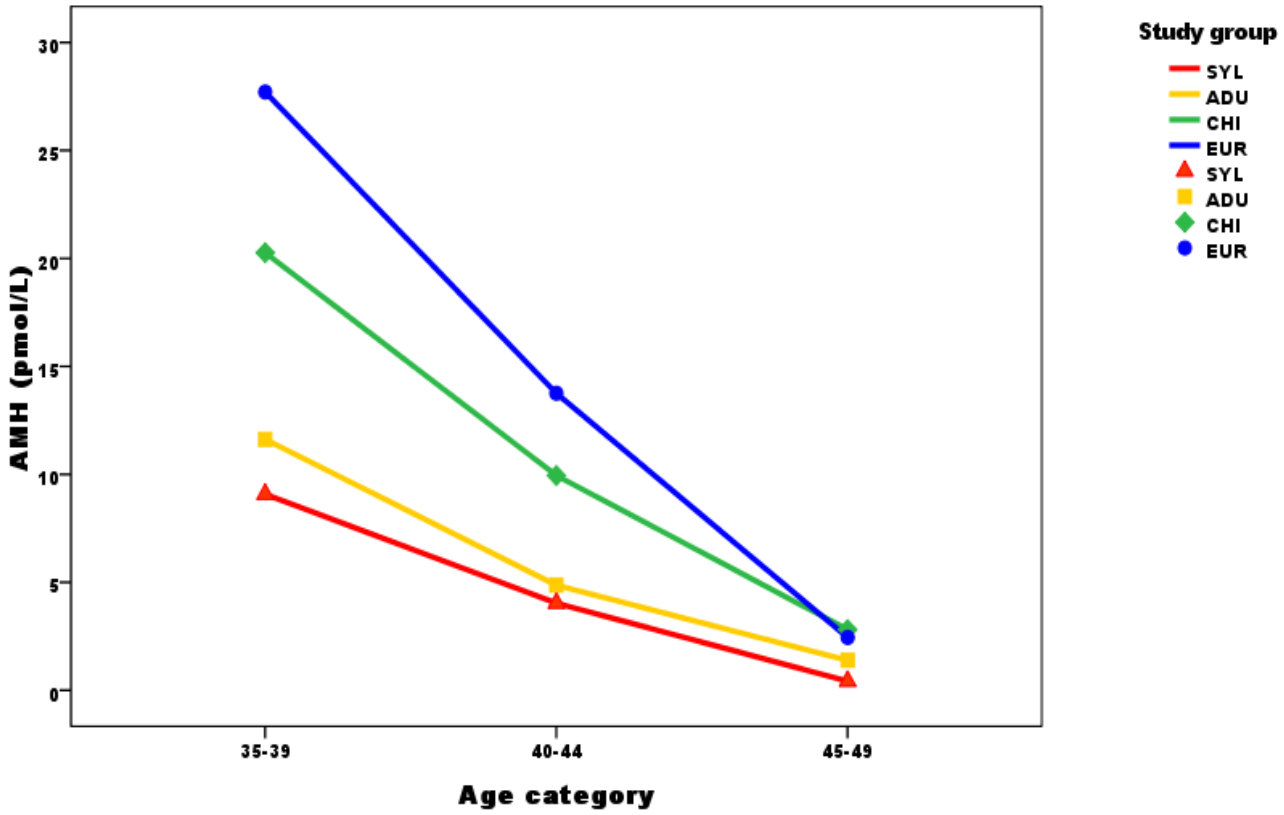


*Figure indicates trend only

Figure 5.4.9 Mean FSH by age category across the study groups (SYL, ADU, CHI and EUR)

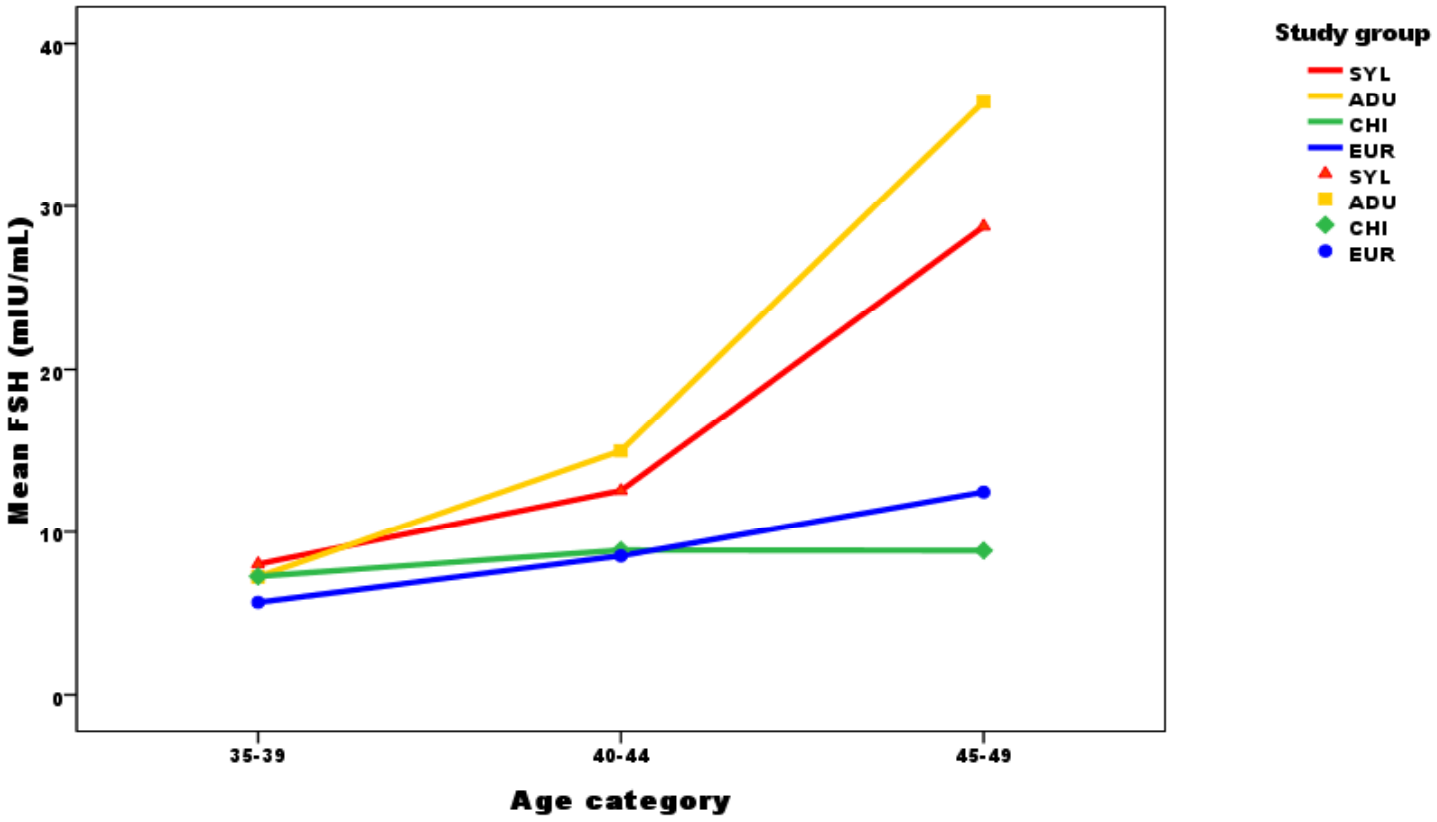


*Figure indicates trend only

CHAPTER 6

DISCUSSION, CONCLUSION AND IMPORTANCE OF THE STUDY

In this study, variability of reproductive ageing and ovarian reserve among migrant Bangladeshi women has been examined by comparing age-related reproductive hormone levels between women in their community of origin (Bangladesh) and their host community (UK). The present study was carried out with the intention of examining reproductive ageing and ovarian reserve among women living and/or developing in different environments, and focused on four different groups: 1) sedentee Bangladeshis who grew up and live in a more adverse environment where they are exposed to higher rates of infectious and parasitic diseases (SYL), 2) adult migrant Bangladeshis who grew up in the same environment as the sedentees but migrated as adults to a better environment in London (ADU), 3) child migrants who migrated to the UK aged less than 16, and therefore grew up and live in a better environment in London (CHI), and 4) white European women who grew up and are living in the same environment as Bangladeshi child migrants in London (EUR). The study compared three different reproductive hormones (inhibin B, anti-müllerian

hormone (AMH) and follicle-stimulating hormone (FSH) between groups, levels of which represent estimates of ovarian reserve.

In this study, I tested three related hypotheses:

1) There is inter-population variation in ovarian reserve depending on environmental conditions during development.

2) Moving to a better environment during adult life does not affect age-specific ovarian reserve.

3) The childhood environment has an impact on age-related ovarian reserve in later life.

From these Hypotheses, I predicted three outcomes:

1) Bangladeshi groups (ADU and SYL) who spent their childhood in Bangladesh will have a lower age-specific ovarian reserve compared to EUR;

2), Both SYL and ADU will have a similar age-specific ovarian reserve;

3) CHI will have an age-specific ovarian reserve that is higher than ADU and SYL, but comparable to EUR.

The findings of this study in supporting these predictions are exceptionally promising and give an insight into the impact of the environment on reproductive ageing and ovarian reserve, as well

as an overview of ethnic variability in menopause among the UK population. This chapter discusses the findings of the present study against previous research and sheds lights on possible explanations for the findings in the context of the proposed hypotheses.

6.1 Hypothesis I: Inter-population variation in ovarian reserve and environmental conditions during development

As discussed in Chapter 2, life history theory posits that division of available energy is divided into three competing compartments of growth, maintenance and reproduction. Constraints on resources that may arise from nutritional deprivation, energetic and/or immune challenges may therefore have a potential impact on reproductive function (Ellison, 2001).

The causes and significance of variation in ovarian function between populations have not been well understood, but reproductive ecologists have suggested that this variation may be related to developmental factors that occur as a result of differences in energy availability during growth and development (Ellison, 1996; Worthman, 1999). The variability of energy availability leads to different maturational tempos and sets a different level of regulation of the hypothalamic–pituitary-gonadal

(HPG) axis to maintain adult reproductive function (Ellison, 1996; Lipson, 2001; Vizthum, 2001).

The developmental environment during early life and the influence of ecological factors during the life course are important for a female. For example, environmental stressors such as nutritional insufficiency and heavy energy output influence female reproductive hormonal levels (Jasienska, 1996; Ellison et al., 1989; Panter-Brick, 1992; Panter-Brick & Ellison, 1994). Several studies have suggested that there are significant differences in indices of ovarian function between populations depending on these kinds of energetic stressors. Studies comparing trajectories of ovarian function by age (Ellison et al. 1993) and progesterone and oestradiol profiles of widely diverse populations revealed inter-population differences (Danutra et al., 1989; Bentley et al., 1990; Ellison et al., 1993; Panter-Brick et al., 1996; Vitzthum et al., 2002).

The data on variation of energy availability mostly concentrated on nutritional and energetic factors (Ellison et al., 2001). It is evident that immunological challenges can cause deprivation of energy in the other life history compartments. Although the evolution of the life history and of disease resistance is generally linked biologically, fighting off diseases requires resources and these resources are also required by other

components of bodily energy. The resources or costs required to fight immunological challenges must therefore be diverted from the other life history compartment of fitness such as growth and reproduction.

Ample evidence points to epidemiological factors as inexorably linked to variation in chronic energy availability. Several authors (Tanner, 1992; Solomons et al., 1997; Moore et al., 2001; Campbell et al., 2003; Panter-Brick et al., 2004) suggested that chronic illness has a negative impact on growth and maturation. Frequent or chronic illness provokes persistent stimulation of the body's immune defences (Zuk and Stoehr, 2002). For example, in chronic illness, persistent energy deprivation can result from constant provocation of the immune system through stimulating inflammatory response to produce antibody and/or lymphocyte activation to prevent infections. From a life history perspective, slower growth, delayed maturation or early reproductive ageing may occur due to energy constraints resulting from excessive investment of energy to combat chronic illness and maintenance of bodily activity (Sheldon and Verhulst, 1996). Therefore, these energy trade-offs can play an important role in the establishment of growth patterns and strategies for the future.

From an epidemiological perspective, the prevalence of infectious diseases, parasitic infections and helminthic infestations is very high in Bangladesh due to poor health facilities, environmental disasters like floods, lack of hygiene, poor sanitation and poor water supplies. In Sylhet, only 25% of the population have access to the treated water supplied by the Sylhet City Corporation, while the rest use tube well or pond water. Furthermore, only 6% people have access to a proper sanitary latrine, 27.5% use a low cost sanitary latrine, while rest of the population do not have access to sanitary latrines. There are poor drainage facilities and no solid waste disposal facilities. Therefore, latrines become the source of faecal contamination during floods (Ahmed et al., 2010) that expose even affluent groups to higher immune challenges (Chapter 3). Therefore, even though the population analysed in this study were from the middle class of Sylhet town with access to clean water and a sanitary latrine, unhygienic conditions can prevail during seasonal monsoon flooding in Sylhet town where infection and disease are endemic.

Each year post-flood trapped water leave lots of breeding ground for mosquitos and insects which cause vector-borne diseases such as malaria. Water from those sources also enhances the spread of water–borne and diarrhoeal diseases (Ahmed et al., 2010). Moreover, the humid climate facilitates egg survival and

spread of helminthic infections (Hall and Holland, 1992). The widespread infectious disease and parasitic infestation causes both symptomatic and asymptomatic disease morbidity.

The information regarding the district-wise vector-borne disease prevalence and epidemiology in Bangladesh is inadequate due to a lack of rigorous study. According to the Southeast Asian Region Office (SEARO), WHO report 2007-2009, out of 64 districts, malaria is highly endemic in 13 districts, of which 8 are situated in north-eastern region and 5 situated in south-eastern part of the country. Sylhet is situated in the north-eastern part with the malaria prevalence rate ranging between 1 and 5 per 1000 population of whom 75% suffered from *falciparum* malaria between 2007 and 2009 (WHO, 2009). In another study, Haque et al. (2009) reported a malaria prevalence rate of 0.4% in the north-eastern districts of Bangladesh.

Intestinal parasitic infections are common and ubiquitous in Bangladesh, and the prevalence reported in several studies is as high as 87% (Mutalib et al., 1976; Hall and Holland, 1992; Mascie-Taylor, 1996). The commonest of the worms are roundworm, hookworm and whipworm. However, there is inadequate information about the intestinal parasite prevalence rate of Sylhet due to a lack of studies. Gilgen and Mascie-Taylor (2001) undertook a study in three tea gardens in Sylhet and found

a 71% hookworm prevalence rate among female tea-pluckers. They also found that, overall, there was a 90% prevalence rate of intestinal parasites with 27.5% restricted to a single worm, 33.2% had a double worm infection, and 26.5% were infected with all three worms.

Although the latter study was restricted to tea plantation workers who are not the same population that are the focus of study here, our data suggest that the majority of SYL and ADU have suffered from helminthic infestations, while a higher percentage of SYL have suffered from parasitic infections like malaria, leishmaniasis or amoebic dysentery. For example, in this study about 84% of SYL and 72% of ADU (who spent their pre-pubertal life in Bangladesh) have suffered from intestinal parasites (helminths) such as round worm, hook worm and whip worm. In contrast, only 8% of EUR women suffered from intestinal parasites (see Chapter 5). Therefore the data from this study are consistent with previous work in Bangladesh and suggest an adverse environmental condition, and exposure to higher immunological challenges. In contrast, the environment in London has good health facilities, improved sanitation and a clean water supply.

As has been mentioned earlier (see Chapter 4), the Bangladeshi women participating in this study belong to the middle income group in Bangladesh and mostly (77%) perceived

their financial condition as "OK" or better in the context of Bangladesh (see Chapter 5; Table 5.2.5). Although their living standard is not comparable with that of the UK, these women are by no means nutritionally stressed and have no marked seasonal variation in dietary habit. Moreover they have a sedentary lifestyle with low energy expenditure due to not engaging in physical activities. However, they are clearly exposed to greater immunological challenges from parasitic infestations or other infectious diseases. According to life history trade-offs, there is diversion of energy away from growth and reproduction to maintain fitness to combat immune insults. This improvised energy strategy during the developmental (childhood) period could represent an adaptive life history strategy that could affect future reproductive function.

Hormonal data from the present study reveal that across all the age categories, SYL and ADU have lower levels of inhibin B and AMH, and higher levels of FSH compared to EUR (Table 5.4.1, 5.4.2 and 5.4.3). Mean inhibin B and AMH levels across the age categories show that hormone levels differentiate between EUR on the one hand and ADU and SYL in the other (Figure 5.4.1 and 5.4.2). Moreover, the age-related, declining pattern of inhibin B and AMH levels reveals that SYL and ADU reach undetectable hormone levels at an earlier age compared to EUR. Age-related

FSH levels show an increasing pattern with a relatively earlier and quicker rising pattern with age in SYL and ADU compared to EUR (Figure 5.4.3). This finding is also supported by data on recalled age at menopause of postmenopausal women where SYL (46 yrs.) and ADU (47 yrs) experience an earlier menopause compared to EUR (50 yrs) (Figure 5.2.3).

Therefore, the data clearly suggest that hormone levels of EUR are different from both SYL and ADU. Women who grew up and live in Bangladesh (where the environment is more challenging especially in terms of infectious diseases) have a lower age-specific ovarian reserve compared to EUR women who grew up and live in London. The contrasting environments during development appear to have established a different timeline for reproductive ageing and reproductive function in later life. Therefore, the data support the predictions of Hypothesis 1.

Apart from hormones reflecting ovarian reserve, a number of other reproductive characteristics also follow different patterns between ADU and SYL and EUR as outlined further below.

Age, age at menarche and age at menopause

The self-reported mean age at menarche of the Study Sample across the three groups suggests overall that EUR women have an earlier age at menarche (12.8) compared to SYL (13.1)

and ADU (13.3) (Figure 5.2.2). Nunez de la Mora et al. (2007), in their study of younger Bangladeshi women aged 18-35, found that white European women had a mean age at menarche of 13.1, which is comparable but slightly later than women in this study. They also found similar age at menarche in ADU (13.0) and SYL (13.2) groups. On the other hand, Bosch et al. (2008) have reported a recalled median age at menarche for rural girls in Bangladesh of 15.1 years, while Chowdhury et al. (2000) found an age at menarche of 13 years among rural Bangladeshi girls, though these researchers used a median age at menarche instead of a mean age. However, getting the exact age of menarche for Bangladeshi women is difficult, but several studies with small sample sizes indicate an average age at menarche between 12.9 and 15.8 (Bosch et al. 2008; Haq, 1984; Chowdhury et al., 1977; Aziz and Maloney, 1985). However, the reported age at menarche of the girls in all of these studies mostly is for those from a rural background.

There are differences in several socioeconomic determinants between rural and urban populations in Bangladesh, and 55% of rural households are living below the poverty line (Hossain and Sen, 1992; BBS 2002). In contrast, the data reported in the study here are from the urban middle class who are comparatively higher in all aspects of socioeconomic and nutritional status than

that of the rural. Several studies have suggested an urban-rural variation in age at menarche (Laska-Mierzejewska et al., 1982; Pasquet et al., 1999) reflecting the same differences that we find here with urban, affluent girls having an earlier age at menarche.

Age at menarche is one of the milestones of the female reproductive event and is a proxy for female reproductive maturity, which is generally indicated by first menses. The timing of this life history event is crucial, as variation of age at menarche directly affects future reproductive events and suggests an alteration in energy expenditure strategy from growth to reproductive effort (Stearns, 1992).

Variation in the timing of reproductive maturity between populations can be explained by variation of body mass (Frisch et al., 1971), specifically adiposity which is crucial for triggering menstruation. A wide variation in age at menarche between developing and developed countries can be explained by a discrepancy of BMI between the countries due to nutritional factors. The data from the present study revealed that women of ADU and SYL group had their first menses at similar ages but later than EUR. As women who grew up in Bangladesh face huge immunological challenges that causing disruption in the energy balance between life history compartments, therefore, their growth spurt and maturation may have become delayed.

The self-reported mean age at menopause of the postmenopausal women shows that age at menopause is significantly different between SYL, ADU and EUR. Age at menopause of women is earlier in SYL (46.0 ± SEM 0.75) and ADU (47.0 ± 1.0), compared to EUR (49.6 ± 1.11) (Figure 5.2.3). There are no recent studies on menopause in Bangladesh but a few studies from the past report a mean age at menopause of 42 to 45 years (Caselli et al. 2006, Wood 1994; Karim et al., 1985).

Several studies suggest an earlier mean age at menopause (41-47 years) in less developed countries like Mexico (Beyene and Martin, 2001; Garrido-Latorre et al., 1996), New Guinea (Wood et al., 1985), India (Sarin et al., 1985; Randhawa et al., 1987), Pakistan (Wasti et al., 1993), the Philippines (Goodman et al., 1985), and Cuba (Moreno et al., 1991) compared to developed countries like the United States (MacMahon and Worcester, 1966; Stanford et al., 1987; Whelan et al., 1990; McKinlay et al., 1992), Italy (Parazzini et al., 1992), Spain (Rebato, 1988; Prado and Canto, 1999), and Finland (Luoto et al., 1994). However, the reason for the variability of the age at menopause in different populations is not well understood. Several researchers have suggested that one possible explanation for this variability is associated with early life conditions (Ibanez et al., 2003; Lumey 1992) such as the childhood socioeconomic environment (Lawlor

et al. 2003, Hardy and Kuh, 2005), nutritional factors (Mishra et al., 2007) and behavioural factors such as smoking (Baron, La vecchie and Levi 1990, Sievert, 2006).

BMI and weight status

For BMI, the data show that ADU (27.07) have a higher mean BMI compared to Europeans (25.57) and sedentees (24.89) (Table 5.2.5). A higher percentage of ADU is overweight (53%) compared to SYL (36%) and EUR (27%). Possible explanations for the higher BMI are related to dietary habits, behavioural patterns and lack of physical activity. Traditionally, the dietary habits of the Bangladeshi population include a high carbohydrate and fatty diet (rice and curry). In general, despite the challenges of adjusting to a radically new lifestyle after migration, food habits among migrant Bangladeshis in London, particularly first generation migrants, are similar to those prevalent in Bangladesh. However, changes occur in the consumption of vegetables as the vegetables they used to eat are not easily available in the UK. Therefore, the dietary habit of these first generation migrants becomes high in carbohydrate, fat and protein with less vegetables and fibre. Furthermore, ADU women who migrated to the UK as brides entered into an encapsulated society where cultural norms do not allow them to come out of house alone. Therefore they have very little opportunity to do physical activity or exercise.

Perceived financial condition

SYL and EUR mostly perceived their financial condition as "*OK*", while ADU mostly perceived this as "*struggling*". Contrasts in perceived financial conditions may be due to differences in socio-cultural norms, economic orientation (economy of country) and the ability to earn money. Another possible explanation is that most ADU are not involved in any work due to their low educational qualifications, lack of English proficiency and skills, as they are living on family income earned by their husband, or living on benefits. Conversely, EUR are mostly working and usually live within their incomes. On the other hand, SYL are from a middle class, land-owning population who living in Sylhet town. Economically, Bangladesh is lower ranking compared to the UK; therefore, the middle income family in Bangladesh is better off economically within Bangladesh but not relative to the UK (for example, a middle income family earns more than Tk 10,000/month which is less than £100/month in the UK).

Level of education

Level of education of the women who gave blood is significantly different between groups. In this study, about three-fourths of ADU and more than two-thirds of SYL were educated to less than 10 years of schooling. Conversely, less than one quarter

of EUR (23%) fit in these categories. Possible explanations for the lower level of education of ADU are that, although they are coming from Sylhet town, they have usually grown up in a rural setting which gave them less opportunity for schooling. Moreover, as previously mentioned the Sylhet population mostly marries within its own community and usually marry their kin living in the UK. However, these arrangements usually take place at an early age, which limits their interest in education due to the socio-cultural norms of "women do not need to study after marriage". In addition, after migrating to the UK, women enter into a male dominated social structure, which does not permit them to come out of the house due to fear of racial discrimination and concern of exposure of women to western values and influences.

From the above findings, it is obvious that later life reproductive function is related to conditions experienced during the developmental period. The data suggest that childhood conditions establish a set point for growth and maturation. Women who grew up in adverse environmental conditions in Bangladesh have a slower life history trajectory and end up with a lower age-related ovarian reserve. The findings are consistent with Hypothesis 1 which theorises that where you live and grow up affects levels of reserve ovarian and other reproductive parameters that likely reflect different life history experience.

6.2 Hypothesis II: Moving to a better environment during adult life does not affect age-specific ovarian reserve

According to the developmental hypothesis, populations that develop in environmental adversity with more nutritional, physical and/or epidemiological challenges have lower ovarian function as adults compared to those who developed in improved environment. In their study on young Bangladeshi women, Nunez de la Mora et al. (2007) found that there are no significant differences in baseline salivary progesterone levels between Sylhet sedentees and adult migrant Sylhet women who migrated to the UK as adults and remained living London. The finding of comparable progesterone levels in spite of current contrasting environments, physical activity patterns and BMI, led these authors to suggest that physiological adaptations are established during childhood, determine ovarian function in the future, and remain robust in adult life.

Similarly, the hormone data here reflecting ovarian reserve suggest there are no significant differences between age-related inhibin B, AMH, FSH between SYL and ADU. These two groups have similar age-related declining patterns of inhibin B and AMH and rising patterns of FSH. SYL and ADU also have a comparable age at menarche (13.1 vs 13.3 years respectively), which indicates the similar set point for the maturation, and a

comparable age at menopause (46.0 vs 47.0 years respectively) that reflects a similar tempo of growth that affected their later reproductive life.

Therefore, the data support the second hypothesis that migration to an improved environment after puberty or during adulthood does not have an impact on later life.

6.3 Hypothesis III: Effects of the childhood environment on ovarian reserve

Hormonal data from the present study reveals that CHI has comparable indices with EUR and significantly higher levels of inhibin B and AMH compared to SYL and ADU across all the age categories (Table 5.4.7 and 5.4.8). Furthermore, SYL and ADU reach undetectable hormone levels at earlier ages compared to CHI (Figure 5.4.7 and 5.4.8). Similarly, age-related FSH levels show an increasing pattern with a relatively earlier and quicker rising pattern with age in SYL and ADU compared to CHI (Figure 5.4.9). In contrast, hormonal dynamics and indices of CHI are comparable with EUR. Therefore, moving to the UK during childhood (<16) has a significant effect on Bangladeshi migrants in delaying menopause and prolonging the period of ovarian ageing.

Nunez de la Mora et al. (2007) also found that Bangladeshi migrants who moved before the age of eight years had higher levels of salivary progesterone compared to women who moved from 9-16. In fact, they suggested that the period prior to eight might be a significant threshold for altering ovarian function early in life, as adrenarche (which occurs around the age of eight) plays an important role in maturation. However, in this study no significant difference was found in the hormonal levels of CHI between women who migrated before age eight years and the women who have migrated aged 9-16.

Although the exact mechanism for initiation of adrenarche has not yet been identified, it is proposed that adrenarcheal maturation is a gradual process intrinsic to the adrenal glands that has no distinct trigger. The principal physical consequences of adrenarche are androgen effects, which are related to puberty, but distinct from HPG maturation and function. However, ovarian reserve and later life reproductive function is regulated through the HPG. Therefore, possibly adrenache does not affect ovarian reserve. However, this hypothesis cannot be confirmed from this study due to the small sample size. Further study with larger samples and in depth research in this area is needed to confirm this explanation.

The above hormonal findings are supported by data on recalled age at menopause of the postmenopausal women (Figure 5.2.3). CHI has only one postmenopausal woman with recalled age at menopause of 45 years. On the other hand, average recalled age at menopause is 49.6, 47.0 and 46.0 years for EUR, ADU and SYL respectively. Due to the specifics of Bangladeshi migration history (see Chapter 2), it is hard to find a child migrant in their later life particularly over the age of 50. Therefore, there are no women in the age category 50-54 and 55-59 years. Moreover, most of the CHI (88%) women were below the age of 45 years with only few (12%) within the age range of 45 to 49.

Data on menarcheal age reveals that mean age at menarche of CHI (12.4) is different from SYL (13.1) and ADU (13.3), while mean menarcheal age of CHI is comparable with EUR (12.8) (Figure 5.2.2). This finding reflects contrasting environmental cues during the developmental period that established a different set point for reproductive maturity and a different tempo of growth and maturation. Therefore, the data support the predictions of Hypothesis 3 that the childhood environment affects age related ovarian reserve.

6.4 How developmental conditions affect later life ovarian function

The normal course of endocrine function for reproductive development is regulated through the (HPG axis) (Ganong, 2005; Worthman, 1999). The HPG axis plays two important roles: regulation and maintenance of adult reproductive function, and control of reproductive development and senescence. The timing of reproductive events is a key element in life history strategy and reproductive success (Worthman, 1999). The characteristic features of HPG activity by age are as follows: early gonadal quiescence, activation at puberty that supports adult reproductive function through hypothalamic control, and reproductive senescence due to gonadal ageing.

Metabolic hormones such as insulin, insulin like growth factor 1 (IGF1) and leptin also play important roles in human growth and development. IGF1 promotes childhood growth, has anabolic effects in adults (Poretsky *et al.*, 1985; Samoto *et al.*, 1993), enhances steroid production by granulosa and theca cells in the ovary (McGee *et al.*, 1996), stimulates oocyte maturation and follicular growth (Willis *et al.*, 1996), and also stimulates Gonadotropine Releasing Hormone GnRH secretion in the hypothalamus (Karlsson *et al.*, 1997; Duleba *et al.*, 1998; Poretsky *et al.*, 1999). Similarly, leptin signals to the brain

information about fat stores that are necessary for GnRH and Luteinizing-hormone-releasing hormone (LHRH) secretion for activation of the HPG axis (Mcmillen and Robinson, 2005; Gluckman et al., 2007).

Several studies have suggested that insulin, IGF1 and leptin play a crucial role in establishing the set point for the commencement of pubertal development, the pace of the development surge, and the rise of adrenal activity (at adrenarche) (Apter, 1997; Wilson, 1998; Foster & Nagatani, 1999; Gluckman et al., 2007). It is observed that levels of IGF1 and leptin are significantly increased before puberty. Rising leptin levels have been associated with initiation of puberty in animals and humans, and normal leptin levels are needed for maintenance of menstrual cycles and normal reproductive function. Furthermore, the metabolic hormones play a role in the regulation of adult ovarian function through modulating the HPG axis (Poretsky *et al.*, 1999), apart from their role in regulating the tempo of maturation.

Although the regulation of metabolic activity varies throughout life, the impact of the metabolic hormones on reproductive function is marked during some specific periods of development compared to others (Holt, 2002; Apter, 1997; Suter *et al.*, 2000). In addition, the effect on maturation rate and

ovarian function is dependent on the timing of changes in energetic condition. Therefore, the metabolic axis may play a key role in establishing developmental trajectories through integrating both acute and chronic energetic cues from the environment and coordinating energy allocation for growth, maintenance and reproduction.

Conversely, ovarian ageing is reflected by a decline in the size of the primordial follicle pool overtime. Historically, females have a complete population of primordial follicles at and around the time of birth, which cyclically depleted during the life time due to maturation and atresia of follicles and ending with menopause. Consequently, the ultimate effect of reproductive ageing at menopause is a reproductive "switch off" from complete depletion of the follicular pool or ovarian reserve. Therefore, menopause or ovarian ageing is caused by a combination of: 1) endowment of follicles *in utero*, 2) rate of atresia, 3) rate of recruitment of follicles (antral) each month. The rate of follicular atresia in both the intra- and extra-uterine environments (Ginsburg, 1991), suggests that conditions during developmental life have an important role in influencing age at menopause. The present study reveals that if women move as a child to UK, then ovarian reserve is extended, therefore, menopause will be later. However the

exact mechanism of the variability of the ovarian reserve and ovarian ageing between populations is not yet known.

6.5 Study limitations

Despite the potential findings of the study that give an insight on developmental impacts on ovarian reserve and reproductive ageing, the study has some limitations in making any conclusive comments. The limitations are as follows:

1. As hormonal analyses are quite expensive, we were constrained to have a small sample size, which were not always sufficient to distinguish hormonal differences between age categories. A larger sample size would have given a better picture.

2. In this study, groups were inevitably not well matched for socio-demographic and economic variables due to different economic, cultural and social norms. There are vivid differences in the social and economical structure between Bangladesh and the UK, for example, in educational system, and perception of financial condition.

3. The recruitment techniques that were followed were different for each group. For example, Europeans were recruited through newspapers like *Metro and Camden Journal*, which is a free paper distributed in the morning at the underground, or through web-based advertisements like in "foreignlondon.com"

website. Therefore, educated and working class women mostly had the information about the study and participated in the European group. In contrast, adult migrants were recruited from community centres, and mostly were less educated and not involved with work. Sylheti women in Bangladesh were recruited from the community and, therefore, they represent the general population.

4. Among the study population, 50% of SYL do not know their exact date of birth. Therefore, estimated age was calculated using an event calculator with important memorable national events such as the Bangladeshi War of Independence, Victory Day, the Indo-Pakistan war, major natural disasters (e.g., a cyclone in 1970). The precision of recollection of age was to the nearest whole year.

5. Among the study population, about 41% of the women could not tell their exact age at menarche. To estimate their age at menarche, woman was asked the school year in which her first menstruation started. Overall precision of recall was approximately to half a year (six months). On the other hand, 60% menopausal women could not recall their exact age at menopause. To determine age at menopause, women were prompted to remember their last menstrual period in relation to season of the year, family and political events. Overall precision of the recall was to half a year (six month e.g. women recall their

age at menopause as six month fraction like forty four and a half years).

6. It is well established that early follicular phase serum hormone (inhibin B, AMH and FSH) levels predict ovarian reserve. Therefore, single blood samples during the early follicular phase between days 4-6 of the cycle represent peak inhibin B and baseline FSH levels (Muttukrishna et al., 2004). There is considerable within and between cycle variations in regularly cycling women. Variation in the length of the menstrual cycle and, by implication, its regularity is largely due to variation in the length of the follicular phase. It is well documented that a single blood sample between days 4-6 is highly predictive of all subsequent values within a year. However, examining the relationship of single point hormone levels and within and between cycle variations in women is beyond the context of this study.

7. Although it is well recognised that chronic illness has a negative impact on growth and maturation. Direct reliable measurement of the energetic costs of continuous immune activity and long term consequence on reproductive effort is very difficult to acquire. Information on the experience of recurrent or chronic illness during childhood is difficult to obtain. In future, such issues should be addressed with more initiatives of comprehensive interviewing. For example, a study could collect information about hospitalisations during childhood, the cause any hospitalisation, as

well as the number of episodes of any chronic diseases suffered during childhood, etc.

6.6 Conclusion

From the above discussion, it is evident that there is inter-population variation in age-specific hormones between Bangladeshi, child migrants and European women, and comparable age-specific hormone levels between adult migrant and sedentee Bangladeshi women. The findings of the study agree with the developmental hypothesis that suggests energy availability during the developmental period influences the maturational tempo through hormones which control future reproductive life. Therefore, variability in the environment during the developmental period may set up a different pattern of energy allocation between life history compartments, e.g. development, maintenance and reproduction, which ultimately causes variability in later life reproductive function.

It is quite evident from the literature that overall environmental conditions of the UK and Bangladesh are contrasting. Studies (Ohtsuka et al., 2002; UNEP, 2001) suggest that the environment in Bangladesh is relatively unhealthy due to inadequate health facilities, sanitation and water supply, and increased disease loads like parasitic infections. In contrast, the

UK environment is much improved with good health structures, a clean water supply, good sanitation and hygiene. Thus women who develop in the UK do so in a better and improved environmental condition compared to women in Bangladesh.

In conclusion, it is proposed that energy availability during the developmental period can influence the maturational tempo and levels of adult ovarian function and, eventually, age related ovarian reserve. Following from this, chronic conditions that drain energy like immune system challenges would establish a pattern of energy allocation away from reproductive function. The outcome of such regulation would modulate ovarian reserve depletion increasing the probability of an early menopause.

Finally, this is the first study to show a relationship between the developmental environment and age-related ovarian reserve in older women that compares women living in their country of origin with migrants from the same country living elsewhere. The finding of a later age-related decline in ovarian reserve among child migrants compared to adult migrants and sedentees suggests that childhood environments can have significant effects on reproductive lifespan. Therefore it can be concluded that human reproductive plasticity extends beyond the uterine period. Unfortunately, the exact mechanism to support this assumption is beyond the scope of this study. Therefore, it is recommended that

further rigorous studies should be undertaken with larger sample sizes and more specific methodologies to investigate further the mechanisms behind this assumption.

6.7 Importance of the study

The implication of developmental influences on age-specific ovarian reserve and age at menopause extends beyond consideration of reproductive outcome to other physiological systems. For example, the withdrawal of hormonal support during menopause affects bone density as levels of oestrogen are critical for bone metabolism (Nguyen et al., 1995). Calcium is an essential requirement of bone formation and lowered oestrogen levels associated with menopause reduce the efficiency of calcium absorption by the gut.

Moreover, the inhibin and activin ratio, which plays a crucial role in maintaining balance between bone resorption and bone loss, is imbalanced during menopause and results in increased bone loss or osteoporosis. It is suggested that increased bone loss in women following menopause results from a reduction or loss of ovarian hormones like oestradiol and inhibin (Vural et al., 2005). Therefore, a hypothetically earlier age at menopause is associated with an earlier exposure to lower hormonal support and results in

an early beginning of bone loss and osteoporosis that may result in osteoporotic fracture.

Other physiological systems that are affected by adult ovarian hormonal levels include cardiovascular health (coronary heart disease) and possibly reproductive cancers such as breast cancer (Kelsey and Gammon, 1991; Bullbrook 1991; Sowers and La Pietra 1995; Nunez de la Mora and Bentley 2008). Studies have also suggested that an earlier age at menopause is associated with higher cholesterol levels and higher blood pressure (Kok et al., 2006). Some observational studies suggest an earlier menopausal age is also associated with an increased risk of ischaemic heart disease and mortality (Hu et al., 1999, Grady et al., 2002; Rossouw et al., 2002). South Asian populations such as Bangladeshis, Indians and Pakistanis in the UK are known to have a higher prevalence of cardiovascular disease (Gupta et al., 2006; Acheson, 1998).

A relationship between breast cancer risk, age at menarche and age at menopause is suggested by several studies (Key and Pike, 1988; Henderson et al., 1988; Kelsey and Gammon, 1991; Kelsey et al., 1993). Mitotic activity in the epithelium of the breast alveoli are stimulated by both oestradiol and progesterone during menstrual cycles and would be prolonged with a longer reproductive life span (Dickson and Lippman, 1987; Clarke and

Sutherland, 1990). The impact of age at menarche on breast cancer risk is due to following reasons:

1. As the menstrual cycle starts, the breast tissues begin to be exposed to ovarian steroids during this period;

2. Establishment of regular ovulatory cycles is dependent on age at menarche, so that an earlier menarche is associated with an early attainment of regular ovulatory cycles (Apter & Vihko, 1983), which causes earlier exposure to the steroids ; and

3. Age at menarche affect levels of steroid hormones as higher adolescent and adult oestradiol levels are significantly related with early menarche (Vihko & Apter, 1984; Apter *et al.*, 1989).

On the other hand, it is now well documented that age at menopause is associated with breast cancer. Studies suggested that breast cancer risk is raised with a later age at menopause (Leidy 1998; Parazzini et al., 1992). It is observed that the difference of 5 years in a later age at menopause is associated with a 10% increase in postmenopausal breast cancer risk (Willett et al., 1983). The possible explanation for this association is that late age at menopause represents a longer period of exposure to ovulatory menstrual cycles.

Several studies have suggested that South Asian migrants in the UK have a higher prevalence of cardiovascular disease and lower prevalence of breast cancer (dos Santos Silva et al., 2002, McCormack et al., 2004). The findings of the present study confirm the earlier data of Nunez de la Mora et al. (2007) in that child migrant Bangladeshis have an earlier age at menarche. The present study here also found that CHI have a higher age-specific ovarian reserve indicative of a delayed process of reproductive ageing compared to ADU and SYL. In addition, age at menarche and age-specific ovarian reserve is comparable between CHI and EUR. These findings suggest an intergenerational change in reproductive hormone levels among the migrant Bangladeshi women. This has also been observed in other populations who are in a similar intergenerational transition (Kelsey et al., 1993).

Therefore, these observations give an indication that the epidemiology of the disease pattern may have changed in this population. Changing disease patterns are supported by recent epidemiological studies on the South Asian population in the UK that suggests an increase in the prevalence of breast cancer over the last 10 years (Smith et al., 2003). Therefore, this study here on ovarian reserve and reproductive ageing will provide valid information about ethnic differences in menopausal history, which will guide the intergenerational transition of reproductive and

ageing related disease patterns of breast cancer, osteoporosis and heart disease, and will help in developing strategic plans for the prevention of such health-related incidences.

In conclusion it is important to understand patterns of variation in ovarian reserve and reproductive ageing which have extensive implications for female general and reproductive health. The findings here will be of benefit to improve the reproductive health of this ethnic minority population and provide guidance to future research on ovarian reserve and reproductive ageing of South Asian populations in the UK. Furthermore, the data related to age-specific hormonal changes among Bangladeshi women will help to explore reproductive health issues and future guidance of further study at the population level in Bangladeshi migrants in the UK.

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APPENDIX 1

QUESTIONNAIRES

DEPARTMENT OF ANTHROPOLOGY
University College London
Gower Street London WC1E 6BT Tel +(0)207 679 8839 Fax +(0)207 679 8632
Email gillian.bentley@ucl.ac.uk, Web http://www.ucl.ac.uk/athropology

Informed Consent Form

Title of Project: Reproductive aging and symptom experience at midlife among Bangladeshi immigrants, sedentees, and white London neighbors

Name of Volunteer: _____
Address: _____
_____
Tel No: _____

	YES	NO
I understand what is in the information sheet about the study and have my own copy		
I have had the opportunity to ask questions and discuss the study		
I have received satisfactory answers to all my questions		
I have received sufficient information about this study I have spoken to: _____		
I know what my part will be in the study and I know how long it will take		
I consent to give a blood sample for this study		
I consent to wear a hot flash monitor		
I have been told if there are possible risks		
I know that the UCL Ethics Committee has approved this study		
I understand that any personal information is strictly confidential: I know the only people who may see information about my part in the study are the research team		
I understand that my personal information may be stored on a computer but will be confidential, secure, and will comply with the 1998 Data Protection Act		
I freely consent to be a subject in the study. No-one has put pressure on me		
I know that I can stop taking part in the study at any time		
I agree to the publication of results of this study in appropriate outlets		
I know that if there are any problems, I can contact either Dr. Bentley, Dr. Muttukrishna at 02076796062, Ms Sharmeen at 07946 935704 or Dr. Khurshida at 07828495521		

Comments or Concerns during the Study
If you have any comments or concerns you should discuss these with the Principal Researcher. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should email the Chair of the UCL Research Ethics Committee (gradschoolhead@ucl.ac.uk) or send a letter to: The Graduate School, North Cloisters, Wilkins Building, UCL, Gower Street, London WC1E 6BT who will take complaint forward as necessary.

Signed: ……
Date:……
Full name in Capitals: ……..
Witness's Signature: ……
Date:……
Full name in Capitals: ……

ADMISSION SCREENING QUESTIONNAIRE

Date of interview:_____/_____/_____

Language of interview:_____

Recruitment
place:_____

Referred
by:_____

Researcher:_____

WOMEN WILL NOT BE ELIGIBLE TO PARTICIPATE IN THE PROJECT IF THEY ARE:
-AGE <35, >59
-PREGNANT
-BREASTFEEDING
-USING STEROID CONTRACEPTION (PILL/ IMPLANT) OR OTHER STEROID MEDICATION
-HAVE THYROID PROBLEMS
-HAVE HAD A HYSTERECTOMY
-HAVE HAD AN OVARIECTOMY
-HAVE HRT IN <6 MONTHS
-PARENTS NOT BORN IN UK

1. Name _____

2. Date of birth? Day _____ Month _____ Year _____

3. Home address: _____

4a. Home telephone _____

4b. Mobile number: _____

5. Where were you born? (Village) (Town/City) County _____

Where did you grow up? (Village) (Town/City) County _____

6. If not UK born, year of migration to UK_____

7. Age when migrated to the UK_____

8. Mother's place of birth: (Village) (Town/City)

9. Father's place of birth: (Village) (Town/City)

10. Have you been menstruating in the past 6 months? ( ) yes ( ) no

If the answer is NO, are you menopausal? ( ) yes ( ) no

If the answer is NO, do you have any medical condition/s that you know which caused you stop menstruating?

( ) yes ( ) no, if the answer is YES please specify _____

11. If you are still menstruating, are your menstrual cycles:

( ) Regular ( ) Irregular () More frequent () Less frequent

12. How many days are there from the beginning of one menstrual cycle to the beginning of another?

( ) < 26 days ( ) 26-32 days ( ) > 32 days

13. When was your last menstrual period (months ago and years ago)

Days/Weeks _____ Months _____ Years _____
( ) Don't know

14. Current Marital status
( ) Single ( ) Married ( ) Divorced ( )Separated ( )Widowed
( )Cohabiting/Partnership

15. Are you currently doing anything to avoid or delay pregnancy (either using a device such as IUD or other technique, modern or traditional, such as rhythm method)?
( ) yes ( ) no

16. If yes, what type?

( ) contraceptive pill (or Implant) ( ) injection ( ) IUD ( ) condom ( ) rhythm
( ) breastfeeding ( ) withdrawal ( ) herbs ( ) abstinence ( ) Tubal Ligation
( ) other (please specify) _____

17. If you have ever used oral contraceptives, hormonal injections or implants, please indicate the date you stopped using them

_____

18. If you are not currently using birth control, do you plan to start within the next three months?
( ) yes ( ) no

19. Have you been breastfeeding in the last 6 months?
( ) yes ( ) no

21. Have you had any ovaries removed (ovariectomy)?
( ) yes ( ) no

22. Have you had your uterus removed (hysterectomy)?
( ) yes ( ) no

23. Have you ever been given treatment for fertility or other medical condition?
( ) yes ( ) no

24. Are you diabetic? ( ) yes ( ) no ( ) do not know

25. Do you have any thyroid-related problems?
( ) yes ( ) no ( ) do not know

26. Do you have any ovarian cysts or suffer from polycystic ovarian syndrome (PCOS)?
( ) yes ( ) no ( ) do not know

27. Have you ever been diagnosed with any diseases that might impair your fertility (e.g. chlamydia, syphilis)?
( ) yes ( ) no

28. Have you ever been diagnosed with infertility?
( ) yes ( ) no

29. Have you taken any medications during the past three months?
( ) yes ( ) no

If yes, how many kinds (including alternative medicines ie homeopathic, herbal medications etc.)

Name of medicine/**Why taken**	**Have you taken this medicine in the last 2 weeks?** Yes/No
1	
2	
3	
4	

30. Will you be away from London in the next couple of months?_____

31. Where did you hear about the project?

32. Do you know of anyone who might like to participate in this project (friends, family members, neighbours)? Can you give us their contact details?

Name	Contact Address	Telephone / Mobile number

Observations/Comments: (Ask the participant whether she is interested to put on a hot flash monitor and/or blood draw)

	YES	NO
Blood draw		
Hot flash monitor		

MQ PART 1 **ID Code**

Interviewer _____

Date _____/_____/200__

Language in which the interview was carried out (tick) Sylheti/Bangla/English

Place of Interview _____

Recruitment place/ referred by _____

Comments/Observations_____

We would like to ask some general questions about yourself first.

I. PERSONAL INFORMATION (please specify)

1.1 What is your marital status now?

( ) single ( ) married ( ) separated ( ) divorced ( ) widowed

1.2. If ever married, how old were you when you were first married? _____

1.3. What is your husband's age (give an approximate age if you do not know his exact age.)

_____

The questions in this and following sections (II, III, and IV) will allow us to match individuals in our sample according to various socio-economic, educational and employment characteristics.

II. SOCIOECONOMIC INFORMATION

2.1 What type of accommodation does your household occupy?
( ) house
( ) flat
( ) other, please specify_____

2.2 Does your household own or rent accommodation?
( ) Owns
( ) Rents

If you rent, who is your landlord?
( ) Council / Local authority
( ) Private landlord or letting agency

2.3 Does your household own a car?
( ) yes ( ) no

2.4 How many members are there *permanently* living in your household? _____

Please indicate their relationship to you and their ages:

Relationship to you	Age
1.	
2.	
3.	
4.	
5.	
6.	
7.	

Others _____

2.5In terms of your current financial situation are you
( ) Struggling
( ) OK

( ) Comfortable
( ) Well off
( ) other (please describe) _____

III. EDUCATION INFORMATION

3.1 Have you ever attended /are you attending school?
( ) yes If yes, please specify in which country_____
( ) no

3.2 What is the highest class in school/ college you have completed? _____

3.3 If presently married, has your husband ever attended school?
( ) yes If yes, please specify in which country_____
( ) no

3.4 What is the highest class in school/ college your husband/partner has completed?_____

3.5 What language do you usually speak at home with your spouse or other adults in the house?
( ) mainly English
( ) mainly mother tongue, (please specify) _____
( ) English and mother tongue equally
( ) other language (please specify) _____

3.6 What language do you speak at home with your children?
( ) mainly English
( ) mainly mother tongue
( ) English and mother tongue equally
( ) other language (please specify) _____

IV. EMPLOYMENT INFORMATION

4.1 Have you ever worked outside the home?
( ) yes ( ) no

4.2 Have you ever worked from home (e.g. dressmaking, child minding etc.)?

( ) yes, please specify_____
( ) no

4.3 What is your current occupation? *Include self-employment, volunteer work.*

_____

4.4 If presently married, is your husband currently

( ) Working ( ) Unemployed ( ) Retired ( ) Other (please specify) _____

4.5 What is your husband's most recent occupation? _____

The questions in the following section concern the history of migration of your family and yourself. This will give us an idea of the type of environment you lived in at different stages of your life.

V. MIGRATION

5.1 What was the purpose of your migration from Bangladesh, and who moved with you to settle in UK at the time of migration?
( ) economic
( ) education
( ) accompany family
( ) marriage
( ) other (please specify) _____

( ) who? _____

5.2 Are there any other member(s) of your family living in Bangladesh?
( ) yes ( ) no
If the answer is YES, please specify who is living in Bangladesh and why?

_____

VI. PHYSICAL ACTIVITY

6.1 Do you practice any sport/ do any exercise?
( ) yes If yes, how often (times/week)? _____
( ) no

6.2 If yes, which type?
(Please specify) _____

6.3 Do you walk continuously for more than 20 min on a daily basis?
( ) yes ( ) no

6.4 What types of work do you usually perform at home and how long these take?

Code	Types of work	Daily	Times a week	Time spend
6.4.1	Washing clothes by hand			
6.4.2	Washing clothes in machine			
6.4.3	Mopping floors			
6.4.4	Vacuuming house			
6.4.5	Dusting House			
6.4.6	Cleaning bathrooms			
6.4.7	Looking after children			
6.4.8	Looking after old relatives			
6.4.9	Preparing and cooking food			
6.4.10	Taking kids to school			
6.4.11	Shopping/buying groceries			
6.4.12	Visiting Friend/family/neighbors			
6.4.13	Child minding			

Other (please specify) _____

Time spend _____
The questions in the following section relate to your general health and the clinical history of your family. This information will provide a context in which to interpret the results about your reproductive aging

VII. GENERAL HEALTH

7.1 Thinking back **over the past two weeks**, have you ever been bothered by any of the following? Please indicate the extent to which you are bothered over the past two weeks by any of these symptoms. [Check any that apply and feel free to add comments on the table

Code	SYMPTOMS	Not at all	A little	Quite a bit	Extremely	Score 0-3
7.1.1	Aches/stiffness in joints					
7.1.2	Backaches					
7.1.3	Bloating					
7.1.4	Night sweats					
7.1.5	Diarrhea					
7.1.6	Constipation					
7.1.7	Difficulty in concentrating					
7.1.8	Dizzy spells					
7.1.9	Feeling blue or depressed					
7.1.10	Headaches					
7.1.11	Hot flushes					
7.1.12	Irritability					
7.1.13	Lack of energy					
7.1.14	Leg cramps					
7.1.15	Loss of appetite					
7.1.16	Mood swings/mood changes/crying spell					
7.1.17	Nervous tension					
7.1.18	Numbness in arms or legs					
7.1.19	Persistent cough					
7.1.20	“Pins and needles” or “crawlies”					
7.1.21	Rapid heartbeat					
7.1.22	Shortness of breath					
7.1.23	Sore throat					
7.1.24	Trouble sleeping					
7.1.25	Upset stomach					
7.1.26	Pressure or tightness in head or body					

Other (please specify) _____

This section asks questions about puberty, periods and pregnancies (if any). We realize the details may be difficult to remember but we would be grateful for the best estimates rather than “do not know”. If you have difficulty, remembering the age at which events occurred it sometimes helps to try to think what class you were attending at school when the event took place. This information will help us to interpret your hormonal profile.

VIII. REPRODUCTIVE HISTORY INFORMATION

8.1 What words would you use to **describe Menstruation**?

_____

_____

_____

8.2 What words would you use to **describe Menopause**?

_____

_____

_____

8.3 Do you have any reason to believe you are entering to Menopause?
( ) Yes ( ) No

Why? _____

8.4 With whom have you talked about menopause?

	Yes	No
Doctor		
Mother		
Daughter(s)		
Son(s)		
Friends		
Husband/Partner		
Neighbors		
Sister (s)		
Cousin sister (s)		
Sister in Laws		
Daughter in laws		
None of the above		

Other (please specify) _____

8.5 What do you think is the normal age of menopause? _____

8.6 When you think about your own age at menopause, do you think you are?
( ) On time
( ) Late
( ) Early
( ) Other (please specify) _____
8.7 How do you think a woman feels during menopause transition?

_____

8.8 Do you know how old your mother was, when her periods stopped completely?
( ) Do not know

( ) yes, please indicate _____years

( ) has not stopped yet and she is _____ years old

8.9 How sure are you of your mother's age at menopause? ( ) Not sure ( ) Very sure

8.10 Did she talk with you about her menopause? ( ) Yes ( ) No

8.11 If yes, what did she tell you about it?

_____

_____

_____

8.12. Did your mother have hot flushes? ( ) Yes ( ) No ( ) don't know/ can't tell

8.13 If YES For how many years did your mother have hot flushes? _____

The following pertains specifically to the experience of hot flushes.

8.14 Have you ever experienced a hot flush (circle one)?

( ) Yes ( ) No ( ) don't know/ can't tell

8.15 How frequently do you have hot flushes (currently):
( ) rarely (less than once/month),
( ) once/month,
( ) twice/month,
( ) once/week,
( ) Twice/week,
( ) 3-4 times/week,
( ) once/day,
( ) twice/day,
( ) 3-4 times/day,
( ) 5 + times/day

8.16 Is there anything you do during the hot flush that makes you fell better?
_____

_____

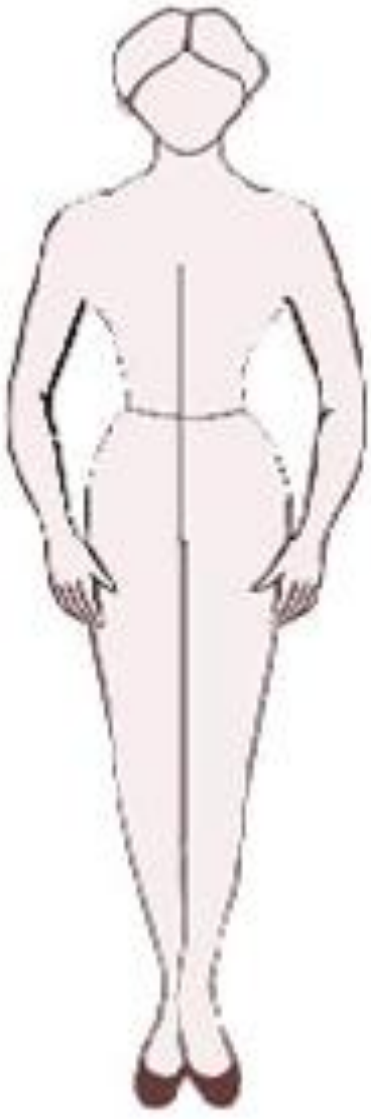
8.17 Is there a time of day when your hot flushes are more severe? ( ) yes ( ) no

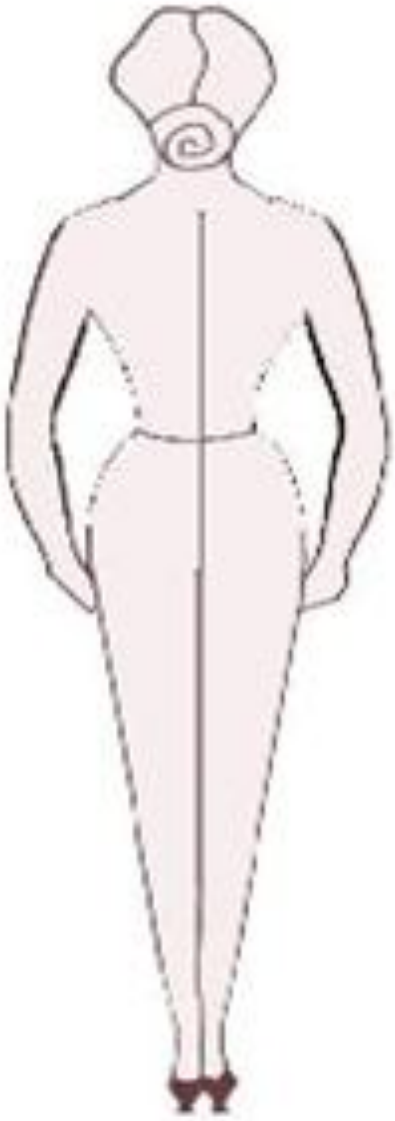
If yes, list when _____

8.18 When you have a hot flash, do you have any of the following feelings? Check if yes.

Code	Feelings	Yes
8.18.1	Heat	
8.18.2	Burning sensation	
8.18.3	Sweating	
8.18.4	Flushed	
8.18.5	Pressure in head	
8.18.6	Pressure in chest	
8.18.7	Change in heart rate	
8.18.8	Change in breathing rate	
8.18.9	Anxiety	
8.18.10	Feel ill/nauseous	
8.18.11	Chills/clamminess	
8.18.12	Embarrassed	
8.18.13	Depressed	
8.18.14	Suicidal	

8.19 On the figures below, note **where** you first feel the hot flush when it is starting (mark with an "X"). Use **arrows** to show where the feeling spreads as the hot flush develops





IX. FOOD FREQUENCY QUESTIONNAIRE

9.1. Which of the following do you eat and how often?

Food type	Raw	cooked	Daily	Weekly	fortnightly	Monthly	occasionally	Never
Chick peas								
Dried peas								
Green peas								
Red lentils								
Mung beans/Green gram								
Broad beans								
Dried bean								
Chick pea flour								
Red Kidney beans								
Garbanzo beans (chola)								
Sweets (including candy bar chocolate, with nuts)[1]								
Carrots								
Broccoli								
Tomatoes								
Cucumber								
Cauliflower								
Whole grain rye bread								
White wheat bread								
Special dietary products (gluten free)								
Cereal dishes (muesli, pancakes)								
Sprouts								
Pumpkin								
Cabbage								
Berries (loganberry, strawberry etc.)								
Fennel								
Ginger								
Turmeric								
Brussels sprout								
Any soy product								
Nuts and seeds								

Thank you very much for your participation and support in helping us with this study. We greatly appreciate your help and hope you have felt able to answer the questions without too much trouble. The information you have given will help us study what factors may influence hormone levels and how these may affect women's health.

If there is anything further you would like to add or comment upon please let us know.

[1] Buter daal er halwa (Garbanzo bean paste), laddu (made with chickpea flour)

MQ PART 2 **ID Code** | | | |

Interviewer _____

Date _____/_____/200__

Language in which the interview was carried out (tick) Sylheti/Bangla/English

Place of Interview _____

Recruitment place/ referred by _____

Comments/Observations_____

X. GENERAL HEALTH

10.1 Do you have any long-term illness, health problem or disability?
( ) yes ( ) no

If yes, please specify_____

10.2 Do you think it limits your daily activities or the work you can do?
( ) yes ( ) no

If yes, please specify_____

10.3 Have you had any illnesses in the last 6 months?
( ) yes ( ) no

If yes, what kind? _____

10.4 Have you taken any medications during the past 6 month?
( ) yes ( ) no

If yes, Was it ( ) Allopathy ( ) Homeopathy ( ) Kabiraji/ Herbal

10.4 b Please state how many kinds (including homeopathy/kabiraji)

Name of medicine	Have you taken this medicine in the last 2 weeks?
1	
2	
3	
4	
5	
6	
7	

10.5 Have you ever been suffered from any of these diseases?

	Name of the disease	Yes	No	Don't know	When
10.5.1	Mumps				
10.5.2	Chicken Pox				
10.5.3	Para Typhoid				
10.5.4	Typhoid				
10.5.5	Diarrhoea				
10.5.6	Tuberculosis				
10.5.7	Measles				
10.5.8	Whooping cough				
10.5.9	Diphtheria				
10.5.10	Pneumonia				
10.5.11	Anaemia				
10.5.12	Arthritis				
10.5.13	Diabetes				
10.5.14	Heart Disease				
10.5.15	Hyperlipidemias (High Cholesterol)				
10.5.16	Hypertension (High Blood preassure)				

Other (please specify)_____

10.6 Have you ever been diagnosed with a parasitic infection?
( ) yes ( ) no

If yes, what kind?
_____

10.7 Have you ever taken any of the following medications?

	Medications	Never	Currently	In the past
10.7.1	Birth control pills			
10.7.2	Hormone [replacement] therapy			
10.7.3	Anti-depressants			
10.7.4	Blood pressure medication			
10.7.5	Daily, low-dose Aspirin			
10.5.6	Cholesterol medication			
10.5.7	Calcium supplements			
10.7.7	Other vitamin/mineral supplements			

10.8 Thinking back **over the past two weeks**, have you ever been bothered by any of the following? Please indicate the extent to which you are bothered over the past two weeks by any of these symptoms. [Check any that apply and feel free to add comments on the table]

Code	SYMPTOMS	Not at all	A little	Quite a bit	Extremely	Score 0-3
10.8.1	Breast tenderness					
10.8.2	Fluid [water] retention					
10.8.3	Menstrual cramps					
10.8.4	Urinary tract/bladder infection					
10.8.5	Vaginal infection/discharge/thrush					
10.8.6	Loss of sexual desire					
10.8.7	Vaginal dryness					

10.9 Have you been diagnosed with a parasitic infection (ie, amoebas, malaria) in the past 6 months?
( ) yes ( ) no

If yes, What kind? _____

10.10 Do you drink caffeinated coffee? ( ) Yes ( ) No

How many cups of caffeinated coffee do you drink _____per day

10.11 Do you drink caffeinated tea? ( ) Yes ( ) No

How many cups of caffeinated tea do you drink _____per day.

10.12 Do you drink caffeinated soft drinks?

How many cans of caffeinated soft drinks do you drink _____per day.

This section asks questions about puberty, periods and pregnancies (if any). We realize the details may be difficult to remember but we would be grateful for the best estimates rather than "do not know". If you have difficulty, remembering the age at which events occurred it sometimes helps to try to think what class you were attending at school when the event took place. This information will help us to interpret your hormonal profile.

XI. REPRODUCTIVE HISTORY INFORMATION

11.1 How old were you at your first menstruation? _____

State whether it is an exact age or an approximation.

( ) exact ( ) approximation

11.2 Which class where you in when you had your first menstruation (if applicable)?

_____

11.3 Are you still menstruating?

( ) yes ( ) no

11.4 How long does the bleeding normally last?

( ) 0-2 days ( ) 3-5 days ( ) 5-7 days ( ) >7 days

11.5 If you are still menstruating, are your menstrual cycles:

( ) Regular ( ) Irregular () More frequent () Less frequent

11.6 How many days are there from the beginning of one menstrual cycle to the beginning of another?

( ) < 26 days ( ) 26-32 days ( ) > 32 days

11.7 When was your last menstrual period (months ago and years ago)

days/weeks _____ Months _____ years _____

11.8 If you are not menstruating, how old were you when your menstrual cycle ended?

Age _____

State whether it is an exact age or an approximation.

( ) exact ( ) approximation

11.9 How long were your menstrual cycles when you were 20-35 years old (tick one)?

( ) < 26 days ( ) 26-32 days ( ) > 32 days

11.10. Did you ever have cramps with your menstrual periods?

( ) Always ( ) Sometimes ( ) Only when younger ( ) Never

11.11 Have you ever been pregnant?

( ) yes ( ) no (if the answer is **No**, go to question no 2.17)

11.12 How many times have you been pregnant?

_____

11.13 Ho may children do you have? _____

11.14 How old were you when you were pregnant for the first time?
_____

11.15 How long had you been married when you had your first child?

_____

11.16 How old were you when you were pregnant for the last time?

_____

11.17 Did you breast fed your children? ( ) yes ( ) no If Yes,

Could you tell me the length of time spent breast feeding your last child? _____

11.18 In a new couple when do you think is the best time to have their first child? (e.g. within a year, wait a bit etc.)

_____

11.19 What do you think is the ideal birth spacing for a woman?

_____

11.20 What is the ideal number of children for a couple? _____

11.21 In the past two weeks, have you ever had any headaches?

( ) yes ( ) no

11.22 How often do you experience a headache? _____

11.23 Do you have headaches more frequently now than before menopause?
( ) yes ( ) no

11.24 Have you ever been told by a doctor that you are experiencing migraines?

( ) yes ( ) no

11.25 Could you please tell us about the symptoms that you experience during headache?

	Symptoms	Yes	No
11.25.1	Do you experience Bilateral pain (both sides)		
11.25.2	Is the intensity is mild to moderate		
11.25.3	Is the intensity is moderate to severe		
11.25.4	Do you feel any pressing or tightening on the head/forehead		
11.25.5	Does it get worse with physical activity		
11.25.6	Are you sensitive to light or sound		
11.25.7	Do you feel nauseous / sick		
11.25.8	Does it last minutes		
11.25.9	Does it last hours		
11.25.10	Does it last days		

This section will be used to record anthropometric measurements like weight, height etc. which will be used for comparative purposes between different groups. It will be easier to do this if you have loose clothes on.

XII. ANTHROPOMETRIC MEASUREMENTS

Name of the anthropometric measures	Measurement	BMI	WH Ratio
A. Height (cm)			
B. Weight (kg)			
C. Tricep skin fold measurement(cm)			
D. Waist measurement(cm)			
E. Hip measurement(cm)			
F. Arm circumference			

Thank you very much for your participation and support in helping us with this study. We greatly appreciate your help and hope you have felt able to answer the questions without too much trouble. The information you have given will help us study what factors may influence hormone levels and how these may affect women's health.

If there is anything further you would like to add or comment upon please let us know.

APPENDIX 2

STUDY POPULATION

1. Household member and number of Children

The result of distribution of the women by household member suggests that larger percentage of adult migrants (43%) and sedentee (50%) women's household have more than 5 members, while more than half of the child migrant (56%) women's household have 3-5 household members. On the other hand, household member of the most of the European women (84%) are fewer than 3 (Table 1). These findings are comparable with the UK and Bangladeshi statistics which suggests an average household size of 2.6, 4.7 and 5.2 person per household in European, adult migrants and sedentees respectively (ONS, 2001, BBS, 2005). Mean number of children are significantly ($F_{3, 491}$=22.650, $p<0.001$) different between groups and Post Hoc tests suggests that EUR (1.6±SD2.69) group have significantly lower than SYL (3.2±1.75), ADU (3.9±1.62) and CHI (3.8±4.42). Further analysis suggestes that SYL, ADU and CHI have higher median number of children than EUR (Table 2).

Table 1 Distribution of study population by household member across the groups

Household Member	SYL	ADU	CHI	EUR	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
<3	16 (10.3)	23 (13.3)	4 (7.4)	123 (83.7)	166 (31.4)
3-5	61 (39.4)	76 (43.9)	30 (55.6)	21 (14.3)	188 (35.5)
>5	78 (50.3)	74 (42.8)	20 (37.0)	3 (2.0)	175 (33.1)
Total	155 (100)	173 (100)	54 (100)	147 (100)	529 (100)

Table 2 Distribution of study population by number of children across the groups

Groups	Number of children		
	Mean	SD	Median
SYL (n=157)	3.2	1.75	3.0
ADU (n=168)	3.9	1.62	4.0
CHI (n=52)	3.8	4.42	3.0
EUR (n=118)	1.6	2.69	1.0
Total (n=495)	3.1	2.53	3.0

ANOVA: $F_{3, 491}=22.650$, $p<0.001$
Post hoc: EUR<SYL, ADU & EUR<0.001

2. Education

Education status suggests that the majority of the migrant Bangladeshi women (85%) had less than 10 years of schooling followed by sedentees (65%), and child migrants (47%), while only 28% of the European women had less than 10 years of

schooling, which is quite comparable with the national data of both countries. In contrast, the highest percentage (72%) of white European women had studied for more than 10 years of which 40% had more than 15 years of schooling. This is followed by the child migrants of whom more than 52% had more than 10 years of schooling, while 4% had more 15 years of schooling. Among the sedentees and adult migrants, about 35% and 15%, respectively, had more than 10 years of schooling while only 4.5% and 3.6% had more than 15 years of schooling (Table 3).

Table 3 Distribution of study population by level of education across the groups

Level of Educational	Group				Total
	SYL	ADU	CHI	EUR	
	N (%)	N (%)	N (%)	N (%)	N (%)
Low	102 (65)	147 (84.5)	26 (47.3)	43 (27.9)	318 (58.9)
Medium	48 (30.6)	19 (10.9)	27 (49.1)	49 (31.8)	143 (26.5)
High	7 (4.5)	8 (4.6)	2 (3.6)	62 (40.3)	79 (14.6)
Total	157 (100)	174 (100)	55 (100)	154 (100)	540 (100)

Low level= 0-10 years of schooling,
Medium level= 11-14 years of schooling,
High level= 15 and 15+ years of schooling

3. Economic condition

The economic status data suggests that a similar picture within the study population. About three quarters (75%) of the adult migrant women have never worked outside the home and they mostly remained in the house and look after the children and household while all of the white European women are actively

involved in work (Table 4). For the perceived financial condition, about 39% and 37% of the adult migrant Bangladeshis perceived their financial condition as "struggling" and "OK" respectively compared to 25% "struggling" and 50% "OK" of the white European women (Table 5), while about 60% of the child migrant perceived their financial condition as "OK". The high unemployment, economic inactivity and low educational qualifications, lead the adult migrants to depend on social benefits and housing (Eade et al., 1996).

Table 4 Distribution of study population by employment status across the groups

Employment Status	SYL	ADU	CHI	EUR	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
No	108 (68.8)	130 (74.7)	10 (18.5)		248 (46)
Yes	49 (31.2)	44 (25.3)	44 (81.5)	154 (100)	291 (54)
Total	157 (100)	174 (100)	54 (100)	154 (100)	539 (100)

Table 5 Distribution of study population by perceived economic condition across the groups

Financial condition (N (%)	SYL	ADU	CHI	EUR	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
Struggling	17 (11.0)	67 (38.5)	5 (9.3)	38 (25)	127 (23.7)
OK	59 (38.1)	64 (36.8)	32 (59.3)	76(50)	231 (43.2)
Comfortable	46 (29.7)	38 (21.8)	15 (27.8)	34(22.4)	133(24.9)
Well off	33 (21.3)	5 (2.9)	2 (3.7)	4(2.6)	44(8.2)
Total	155 (100)	174 (100)	54(100)	152 (100)	535(100)

4. Accommodation and Housing

Accommodation and housing data are presented in table 6. Bangladeshis in the UK mostly live in council flats. In ADU group, of the 89% who live in flats, 76% have rented their accommodation, of which 87% have rented from the local authority or council, while 46% and 53% of the child migrant live in flats and houses respectively, 48% own their accommodation and 52% have rented their accommodation with 64% have rented from the council or local authority. On the other hand, white Europeans lived in either houses (44%) or flats (49%) with 51% owned their accommodation and the rest 49% are renting them. However, two thirds (63%) of those who rented houses did so from the local authority or council. Sedentee Bangladeshis in Sylhet are mostly (83%) living in flat and an almost equal percentage (54% vs 46%) either own or rent from a private landlord respectively (Table 6).

Table 6 Distribution of study population by accommodation status across the groups

	SYL	ADU	CHI	EUR	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
House	1 (0.6)	18 (10.3)	29 (52.7)	67 (43.5)	115 (21.3)
Flat	131 (83.4)	154 (88.5)	25 (45.5)	76 (49.4)	386 (71.5)
Other	25 (15.9)	2 (1.1)	1 (1.8)	11 (7.1)	39 (7.2)
Total	157 (100)	174 (100)	55 (100)	154 (100)	540 (100)
Owns	82 (53.6)	41 (23.6)	26 (48.1)	77 (50.7)	226 (42.4)
Rents	71 (46.4)	133 (76.4)	28 (51.9)	75 (49.3)	306 (57.6)
Total	153 (100)	174 (100)	54 (100)	152 (100)	533 (100)
Council		115 (86.5)	18 (64.3)	45 (60.0)	177 (57.8)
Private	71 (100)	18 (13.5)	10 (35.7)	30 (40.0)	129 (42.2)
Total	71 (100)	133 (100)	28 (100)	75 (100)	306 (100)

APPENDIX 3

LABORATORY TECHNIQUES (HORMONE ANALYSIS)

Blood samples are being analysed for FSH, inhibin Band AMH

1. Follicle stimulating hormone (FSH)

Immunoassay for the in vitro determination for follicle-stimulating-hormone (FSH) in serum was performed by COBAS e 411 (Picture below) immunoassay analyser using the electrochemiluminescence immunoassay technique.

Test principal:

The test followed a sandwich principal:

Step1 (1st incubation): 40µl of sample, a biotinylated monoclonal FSH specific antibody, and a monoclonal FSH-specific antibody labelled with a ruthenium complex from form a sandwich complex.

Step 2 (2nd incubation): After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptovidin.

Step 3 : Reaction mixture as aspirated into measuring cell where microparticles are magnetically catured onto the surface of the electrode. Unbound substancesare then removed with ProCell. Application of voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Step 4: Results are determined via calibration curve which is generated by 2 point calibration and a master curve provided via the reagent barcode.

Reagents-working solution

1. Streptavidin coated microparticles 0.72 mg/ml
2. Anti-FSH-Ab- biotin: Biotinylated monoclonal anti-FSH antibody (mouse) 0.5 mg/L & MES buffer 50 mmol/L, pH 6.0
3. Anti-FSH-Ab-Ru $(bpy)^{2+}_3$: Monoclonal anti-FSH antibody labelled with ruthernium complex 0.8mg/L, pH 6.0

Test procedure:

Test was carried out by COBAS e411 analyzer system according to manufacturer's instruction. The system automatically regulates the temperature of the reagent and reaction, as well as measuring and mixing of the reagents. Samples and reagents were got out from the refrigerator and bring to approximate temp 20º-25ºC and was placed on the sample and reagent disk of the analyser.

The analyser automatically calculates the analyte concentration of each sample. Total duration of assay is about 18 minutes. The test can measure the concentration of FSH as low as 0.10 mIU/mL and as high as 200.0 mIU/mL.

2. Inhibin B

This hormone analysis is being performed using ELISA for the quantitative measurement of dimeric inhibin B in human serum manufactured by Diagnostic System Laboratories, Texas, USA (DSL-10-84100). This ELISA is an enzymatically amplified two-step sandwich-type immunoassay.

All reagents were allowed to reach room temperature prior to assay. The preparation of the standards, control and serum was used in duplicate. The desired number of micro-titre plates was chosen and 50 µl of standards, control and serum were added to each well. Twenty five µl each of inhibin B sample buffer A and sample buffer B were added to each well. The wells were then

covered and were incubated in an orbital micro-plate shaker at 300-400 rpm overnight at room temperature. The wells were then washed 3 times with de-ionized water and were blot dried by inverting plate on absorbent material. Fifty µl of inhibin B antibody-biotin conjugate was then added to each well and were incubated on an orbital micro-titre plate shaker set at 500-700 rpm for 1.5 hours at room temperature. The wells were then washed 6 times with wash solution and were blot dry as previously stated. Fifty µl of Streptavidin-enzyme conjugate were added and were incubated for 20 min on an orbital microtitre plate shaker at a rate of 500-700 rpm. Then the microtitre plate was washed 6 times and was soaked in the wash solution for 15 minutes. After that, the wells were washed 6 times and were blot dry as above. TMB 100 µl was added to each well and were incubated on an orbital microtitre plate shaker at a rate of 500-700 rpm for 15 min at room temperature. After incubation, 100 µl of stop solution was added to each well and was absorbance read using a microtitre plate reader at 450 nm. Results were obtained from best fit standard curve by interpolation. The curve serves for the determination of inhibin B concentration in samples was measured at the same time as the standards.

3. Anti-müllerian hormone (AMH)

This hormone analysis was performed using an ELISA manufactured by Immunotech SAS, Marseille, France (A 16507). This ELISA is a two step sandwich-type immunoassay.

All the components of kit equilibrate were kept for 30 minutes at room temperature before use and, after solubilisation of lyophilised components, left for 10 minutes at room temperature before use. Fifty ml of the wash solution was diluted with 950 ml of distilled water and reconstituted with biotinylated monochlonal

antibody and the concentrated calibrator with distilled water according to instructions. Freshly prepared 1500 pM AMH and the 0 pM calibrators were used. The dilution series were as follows:

Calibrator concentration	**AMH**	**Calibrator 0**
150pM	50µl of 1500pM	450 µl
81 pM	200µl of 150pM	170 µl
27 pM	100µl of 81pM	200 µl
9 pM	100µl of 27pM	200 µl
3 pM	100µl of 9pM	200 µl
0 pM	-	200 µl

Step 1: 25 µl of calibrator or serum sample with 100 µl of reaction buffer were added per well and incubated for 2 hours at 18-25º C with shaking. After incubation, the wells were washed.

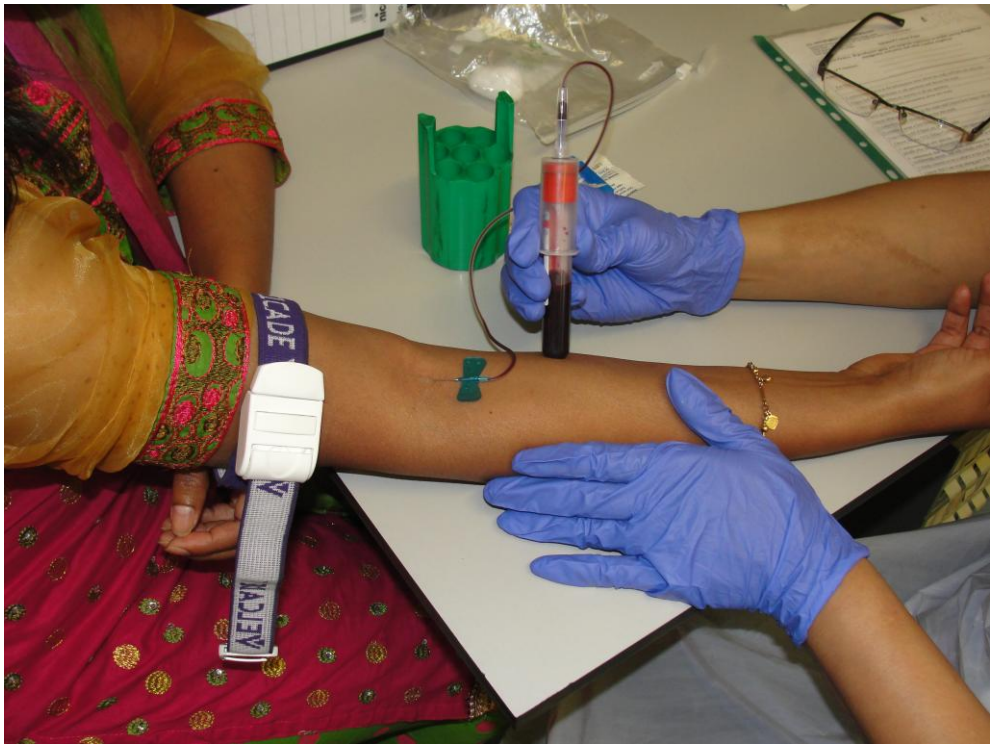
Step 2: Biotinylated antibody 50 µl and streptavidin-HRP were added to each well and incubated for 30 min at 18-25º C with shaking. After incubation, wells were washed.

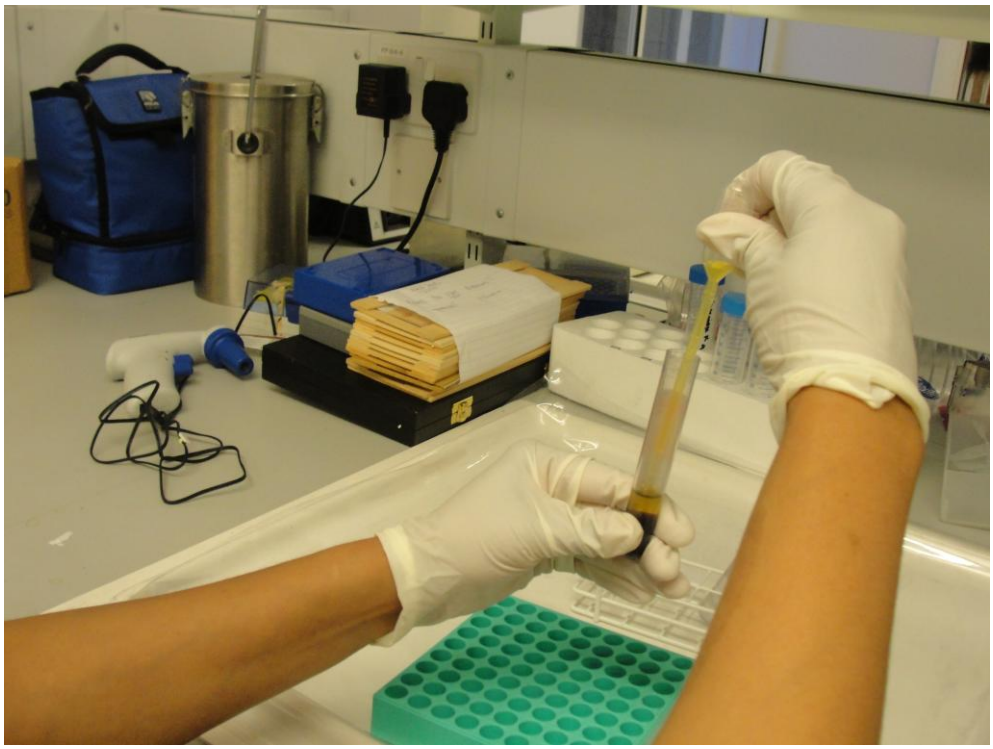
Step3: 100 µl of substrate were added to each well and incubated for 30 min at 18-25º C with shaking. After incubation, 50 µl of stop solution were added and the plate was read at 450 nm.

Results were obtained from calibrator curves by interpolation. The curve provided determination of AMH concentration in samples measured at the same time as the calibrator.

APPENDIX 4

Blood draw and laboratory work





Centrifuged machine

Cobas e 411 Immunoassay Analyzer