European Journal of Obstetrics & Gynecology and Reproductive Biology: X 4 (2019) 100055

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology: X

journal homepage: www.elsevier.com/locate/eurox

Live birth rate is associated with oocyte yield and number of biopsied and suitable blastocysts to transfer in preimplantation genetic testing (PGT) cycles for monogenic disorders and chromosomal structural rearrangements

Jara Ben-Nagi[a,*], Benjamin Jones[b], Roy Naja[c], Ahmed Amer[a], Sesh Sunkara[d], Sioban SenGupta[e], Paul Serhal[a]

[a] Centre for Reproductive and Genetic Health, 230–232 Great Portland Street, London, W1W 5QS, UK
[b] Imperial College London, Du Cane Road, London, W12 0NN, UK
[c] IGENOMIX, 40 Occam Road, Guildford, Surrey, GU2 7YG, UK
[d] Queen's Hospital, Barking Havering and Redbridge University Hospitals NHS Trust, Essex, UK
[e] UCL, Institute for Women's Health, 86-96 Chenies Mews, London, WC1E 6HX, UK

ARTICLE INFO

Article history:
Received 14 March 2019
Received in revised form 19 May 2019
Accepted 23 May 2019
Available online 1 June 2019

Keywords:
Live birth
Preimplantation genetic testing
Monogenic disorders
Chromosomal rearrangements
Oocyte
Blastocysts

ABSTRACT

Objectives: To investigate whether live birth (LB) is associated with oocyte yield and number of biopsied and suitable blastocyst to transfer following preimplantation genetic testing (PGT) for monogenic disorders (PGT-M) and chromosomal structural rearrangements (PGT-SR).
Study design: All couples underwent controlled ovarian stimulation, blastocyst biopsy, vitrification and transfer of suitable embryo(s) in a frozen embryo transfer (FET) cycle.
Results: Of 175 couples who underwent PGT treatment, 249 oocytes retrievals were carried out and 230 FET were subsequently undertaken. 122/230 (53%, 95% CI 47–59) FET resulted in a LB and 16/230 (7%, 95% CI 4–11) have resulted in ongoing pregnancies. 21/230 (9%, 95% CI 6–14) FET resulted in miscarriage and 69/230 (30%, 95% CI 24–36) concluded with failed implantation. Two (1%, 95% CI 0–3) transfers underwent termination for congenital malformation, with no evidence of misdiagnosis by prenatal testing. The relationship between number of oocytes retrieved and number of blastocysts biopsied and suitable embryos to transfer were significant (p = 0.00; Incidence rate ratio (IRR) 1.05; 95% 1.04–1.06; p = 0.00; IRR 1.04; 95%, 1.03–1.06), respectively. The number of oocytes collected (p = 0.007; OR 1.06; 95% CI 1.01–1.10), the number of blastocysts biopsied (p = 0.001; OR 1.14; 95% 95% CI 1.06–1.23) and the number of suitable embryos to transfer (p = 0.00; OR 1.38; 95% CI 1.17–1.64) were all significantly associated with the odds of achieving a LB. There is a 14% and 38% increased chance of a LB per additional blastocyst biopsied and suitable embryo to transfer, respectively.
Conclusions: PGT-M and PGT-SR outcomes are significantly associated with egg yield, number of blastocysts to biopsy and suitable embryos to transfer.

Introduction

Preimplantation genetic testing (PGT) for monogenic disorders (PGT-M) or chromosomal structural rearrangements (PGT-SR) is an alternative to prenatal diagnosis for couples who are inherently predisposed to the transmission of a genetic disorder to their offspring. If couples decide to utilise PGT, it necessitates the use of in vitro fertilisation (IVF), regardless of fertility, to produce embryos to biopsy.

In IVF cycles, it has been shown that there is a strong correlation between the number of oocytes retrieved and live birth rate [1]. As such the number of oocytes can be used as a robust surrogate outcome for clinical success. Although the relationship is not linear, the live birth rate rises with increasing number of oocytes retrieved, up to 15. For successful PGT outcome, it has also been demonstrated that the number of cumulus oocyte complex is significantly associated with the number of embryos biopsied, and the number of suitable embryos to transfer [2].

* Corresponding author.
E-mail address: jara.bennagi@crgh.co.uk (J. Ben-Nagi).

http://dx.doi.org/10.1016/j.eurox.2019.100055

With extended embryo culture resulting in increased rates of blastocyst formation, blastocyst biopsy has been successfully performed in PGT cycles [3,4]. Retrieval of three to six trophectoderm cells from a blastocyst compares more favorably to the removal of 1–2 cells from a cleavage-stage embryo. In a paired randomized clinical trial, day-3 embryo biopsy led to 30% implantation and live birth, in comparison to 50% for unbiopsied controls, which represents a relative reduction of 39% [5]. In contrast, implantation rates were similar for biopsied and control blastocysts [5].

Furthermore, following the advent of vitrification for cryopreservation of embryos, recent systematic reviews and randomized controlled trials have indicated comparable or improved pregnancy rates with frozen embryo transfer compared with fresh embryo transfer [6,7]. Hence, IVF centres carrying out PGT-M, PGT-SR and preimplantation genetic testing for aneuploidies (PGT-A) have adopted this strategy of blastocyst biopsy, cryopreservation and frozen embryo transfer for PGT cases.

To the best of our knowledge, there has not yet been published data to investigate the association between oocyte and blastocyst yield with live birth rate in PGT-M and PGT-SR cycles. The aim of this study was to provide evidence that live birth rate in PGT-M and PGT-SR cycles is associated with the number of oocytes retrieved, blastocysts biopsied and suitable embryos available for transfer. This evidence can assist reproductive medicine specialists and embryologists in counselling women, in order to identify the oocyte and blastocyst thresholds that increase the probability of live birth following PGT.

Material and methods

Patient selection

All couples referred to the IVF Centre for PGT-M and PGT-SR over a four-year period between 2014 and 2017 were included in the study. Following a detailed assessment of the genetic history it was determined whether Karyomapping or array comparative genomic hybridisation (a-CGH) was possible for monogenic or chromosomal rearrangement, respectively.

For PGT-M, the Karyomapping method adopted has been previously described by Natesan et al. [8] and Konstantinidis et al. [9]. Karyomapping was also able to detect chromosomal aneuploidies in the tested embryos that were subsequently considered for transfer. Whilst for PGT-SR, a-CGH has been described elsewhere [10,11] and implemented in the current study.

IVF, blastocyst biopsy and embryo transfer

IVF was commenced once the PGT-M workup was complete for single gene disorder or the genetic laboratory was able to identify the chromosomal rearrangement with a diagnostic accuracy of more than 95% for PGT-SR. All couples underwent controlled ovarian stimulation, blastocyst biopsy, vitrification and subsequent medicated frozen embryo replacement cycle, which has been previously described [12].

Ethics

All patients received genetic counselling. The IVF process including PGT was explained in detail to ensure that all couples were fully informed. Written consent was received for IVF and PGT. No embryo was subjected to research. Thus, this retrospective case series was exempt from ethical approval.

Statistics

Descriptive statistical analysis was described as mean +/− SD. Logistic regression analysis was performed, to evaluate either the probability of a live birth or the probability of at least one suitable embryo when the explanatory/independent variable was either the total number of oocytes, the number of blastocysts or the number of suitable embryos, as appropriate. Age in years was included as an additional explanatory variable in these logistic regression analyses. A receiver operating characteristic (ROC) curve was plotted giving an indication of the diagnostic ability of the explanatory variable in the logistic regression to discriminate between a binary outcome/dependent variable of “live birth” (yes/no) or of “at least one suitable embryo” (yes/no) as its threshold varied. The area under the ROC curve was evaluated to measure how well the explanatory variable distinguished between the two diagnostic groups [e.g. live birth (yes/no)]. Poisson regression was used to evaluate the effect of the number of oocytes retrieved either on the number of blastocysts biopsied or on the number of suitable embryos to transfer, again using age in years as a covariate. All analyses were performed using Stata (StataCorp). 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC) and statistical significance was achieved if $p < 0.05$.

Results

175 couples underwent PGT. 145 (83%) couples had PGT-M whilst 30 (17%) had PGT-SR. 44 (25%) couples had a second or third cycles. 17 (10%) couples underwent embryo batching in order to maximise their egg yield and blastocyst formation rate in view of diminished ovarian reserve and/or previous low response to controlled ovarian stimulation. 249 oocyte retrievals were carried out; 196 (79%) because of single gene disorders and 53 (21%) for chromosomal rearrangement. The mean female age was 33.9 years (SD ± 3.8). Table 1 shows PGT cycle laboratory outcomes and embryo biopsy results.

A total of 230 frozen embryo transfers were carried out. 122 (53%, 95% CI 47–59) resulted in a live birth and 16 (7%, 95% CI 4–11) have ongoing pregnancies. 21 (9%, 95% CI 6–14) frozen embryo transfers resulted in miscarriage and 69 (30%, 95% CI 24–36)

Table 1
PGT cycle laboratory outcomes and embryo biopsy results.

PGT cycle characteristics		95% Confidence Intervals
No. of oocytes	3,149	N/A
No. of metaphase II oocytes	2,672	N/A
Fertilisation rate	72% (1923/2672)	70-74
Blastulation rate	66% (1262/1923)	63-68
Proportion of blastocysts for monogenic disorders (%)	1022/1262 (82)	79-83
Proportion of blastocysts for chromosomal rearrangement (%)	240/1262 (18)	17-21
Proportion of blastocysts suitable for transfer (%)	441/1262 (35)	32-38
Proportion of blastocysts unsuitable for transfer (%)	821/1262 (65)	62-68
Proportion of blastocysts unaffected but aneuploid (%)	203/639 (32)	28-35

PGT = preimplantation genetic testing cycle; No.: number; N/A: not applicable.

concluded with failed implantation. Two (1%, 95% CI 0–3) transfers resulted in termination for congenital malformation, with no evidence of misdiagnosis by pre-natal testing.

Whole group analysis

The relationship between the number of oocytes retrieved and number of blastocysts biopsied, after adjusting for age in years, was significant (p = 0.00; Incidence rate ratio (IRR) 1.05; 95% 1.04–1.06). Similarly, there was a significant relationship between number of oocytes retrieved and the number of suitable embryos to transfer (p = 0.00; IRR 1.04; 95%, 1.03–1.06). Fig. 1 demonstrates the probability of at least one suitable embryo against the number of oocytes retrieved.

The number of blastocysts biopsied was significantly associated with the odds of at least one suitable embryo (p = 0.00; OR 1.61; 95% CI 1.35–1.95) (Fig. 2). The area under the ROC curve was 0.80 indicating a good discriminating power to predict the odds of at least one suitable embryo with increasing number of blastocysts biopsied.

The total number of oocytes collected was significantly associated with the odds of a live birth (p = 0.007; OR 1.06; 95% CI 1.01–1.10) (Fig. 3). The area under ROC curve was 0.59 for the total number of oocytes collected when the binary outcome of interest was whether or not the woman had a live birth.

The total number of blastocysts biopsied was significantly associated with the odds of a live birth (p = 0.001; OR 1.14; 95% 95% CI 1.06–1.23).The number of suitable embryos was also significantly associated with the odds of a live birth (p = 0.00; OR 1.38; 95% CI 1.17–1.64) (Fig. 4). The area under ROC curve was 0.71, which indicates the number of suitable embryos has a moderate discriminating power to identify women who will have a live birth.

Table 2 summarises the inter-relationships between age and associations between number of oocytes retrieved, blastocysts biopsied, suitable embryos to transfer and odds of a live birth for the whole group, single gene and chromosomal rearrangement group.

Single gene disorders

On further analysis of couples embarking on PGT-M, the number of oocytes retrieved was a significant predictor of the number of blastocysts biopsied (p = 0.00; IRR 1.05; 95% CI 1.04–1.05). There was also a significant association between the number of oocytes retrieved and number of suitable embryos obtained (p = 0.00; IRR 1.04; 95% CI 1.03–1.05).

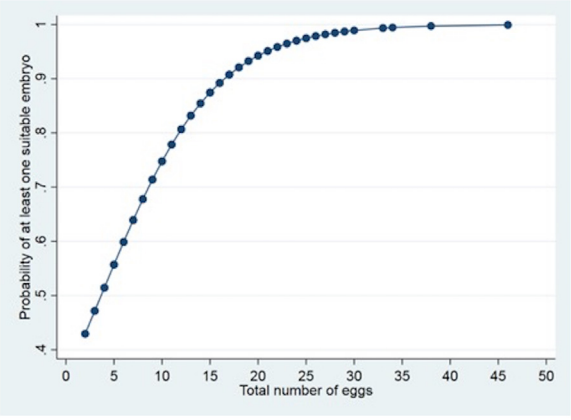

Fig. 1. Graph showing the probability of having at least one suitable embryo to transfer against the number of oocytes collected.

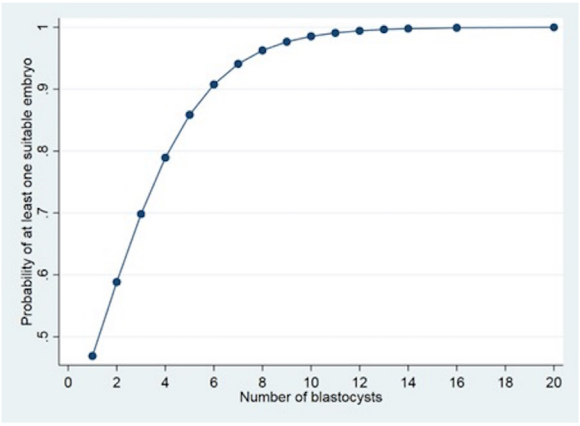

Fig. 2. Graph showing the probability of having at least one suitable embryo to transfer against the number of blastocysts biopsied.

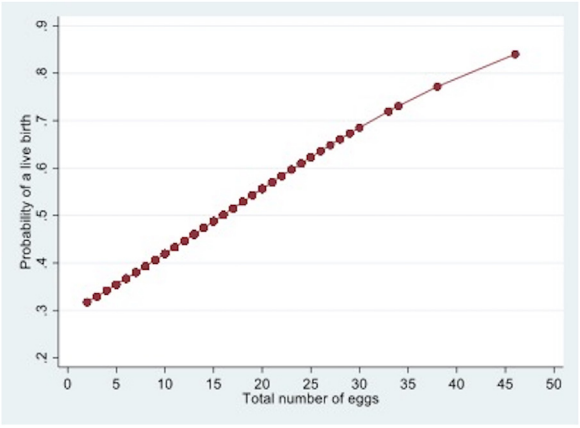

Fig. 3. Graph showing the probability of achieving a live birth against number of oocytes retrieved.

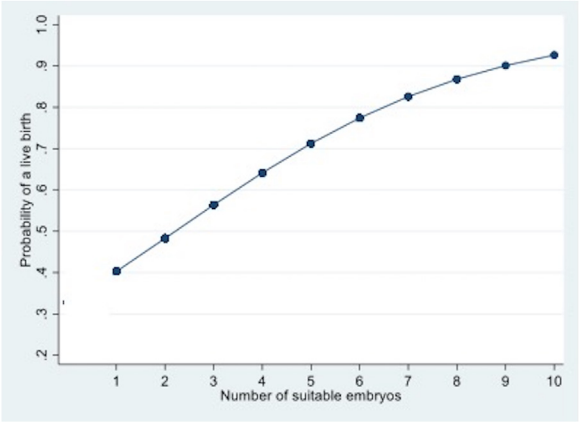

Fig. 4. Graph showing the probability of live birth against number of suitable embryos to transfer.

The total number of oocytes (p = 0.04; OR 1.05, 95% CI 1.00–1.09) (Fig. 5) and the number of blastocysts biopsied (p = 0.01; OR 1.12; 95% CI 1.03–1.22) (Fig. 6) had a significant effect on the odds of a live birth. The number of suitable embryos obtained also had a significant effect on the odds of a live birth (p = 0.004; OR 1.28; 95% CI 1.08–1.51) (Fig. 7).

Table 2
Inter-relationships between age and associations between number of oocytes retrieved, blastocysts biopsied, suitable embryos to transfer and odds of a live birth.

		Age		
		P value	IRR / OR	95% CI
Whole group	Oocytes retrieved vs Blastocysts biopsied	**0.035**	0.98 (IRR)	0.97-0.99
	Oocytes retrieved vs Suitable embryos to transfer	**0.001**	0.94 (IRR)	0.92-0.96
	Blastocysts biopsied vs Suitable embryos to transfer	**0.002**	0.85 (OR)	0.76 – 0.94
	Oocytes retrieved vs Odds of live birth	0.22	0.96 (OR)	0.89-1.03
	Blastocysts biopsied vs Odds of live birth	0.347	0.97 (OR)	0.90 – 1.04
	Suitable embryos vs Odds of live birth	0.76	0.99 (OR)	0.92 – 1.06
Single gene disorders	Oocytes retrieved vs Blastocysts biopsied	0.12	0.99 (IRR)	0.97 – 1.00
	Oocytes retrieved vs Suitable embryos to transfer	**0.00**	0.95 (IRR)	0.93-0.98
	Blastocysts biopsied vs Suitable embryos to transfer	**0.001**	0.96 (IRR)	0.95-0.98
	Oocytes retrieved vs Odds of live birth	0.45	0.97 (OR)	0.90-1.05
	Blastocysts biopsied vs Odds of live birth	0.57	0.98 (OR)	1.02-1.21
	Suitable embryos vs Odds of live birth	0.89	0.99 (OR)	0.92-1.08
Chromosomal rearrangements	Oocytes retrieved vs Blastocysts biopsied	0.27	0.98 (IRR)	0.94-1.02
	Oocytes retrieved vs Suitable embryos to transfer	**0.01**	0.90 (IRR)	0.83-0.98
	Blastocysts biopsied vs Suitable embryos to transfer	**0.009**	0.68 (OR)	0.52-0.91
	Oocytes retrieved vs Odds of live birth	0.45	0.93 (OR)	0.78-1.11
	Blastocysts biopsied vs Odds of live birth	0.57	0.95 (OR)	0.79-1.14
	Suitable embryos vs Odds of live birth	0.68	1.05 (OR)	0.84 – 1.30

CI; Confidence Interval, IRR; Incidence rate ratio, OR; Odds Ratio.
Bold values: Level of significance if $p < 0.05$.

Chromosomal structural rearrangements

For couples embarking on PGT-SR, the total number of oocytes retrieved was a significant predictor of the number of blastocysts biopsied (p = 0.00; IRR 1.09; 95% CI 1.07–1.11). The total number of oocytes collected was a significant predictor of whether there was at least one suitable embryo to transfer (p = 0.02; OR 1.18; 95% CI 1.02–1.36) (Fig. 8). The area under the ROC curve was 0.85. The

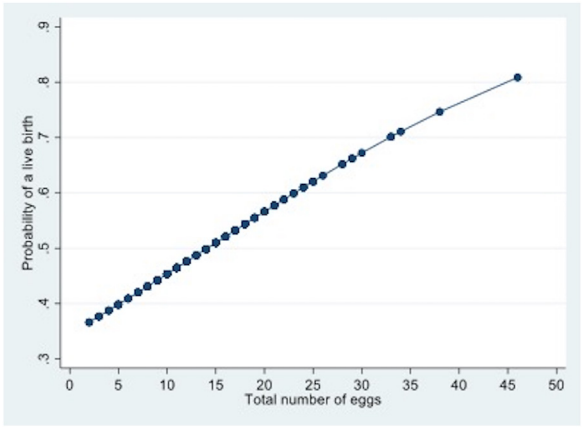

Fig. 5. Graph showing the probability of having a live birth against the number of oocytes retrieved in patients undergoing PGT-M.

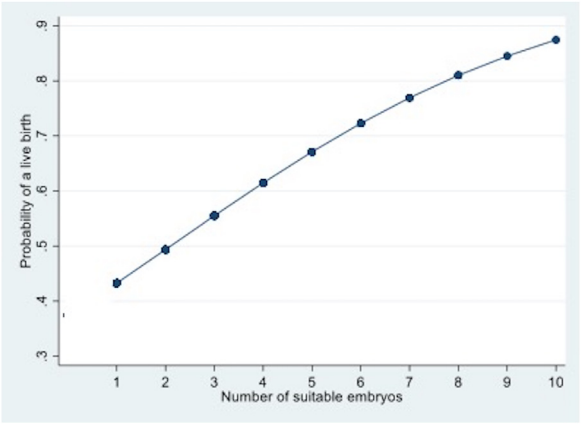

Fig. 7. Graph showing the probability of having a live birth against the number of suitable embryos to transfer in patients undergoing PGT-M.

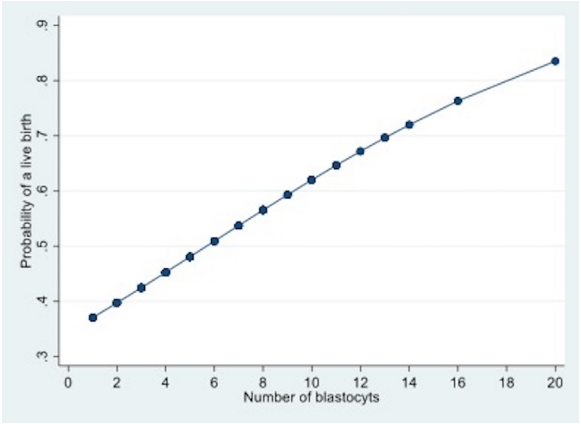

Fig. 6. Graph showing the probability of having a live birth against the number of blastocyst biopsied in patients undergoing PGT-M.

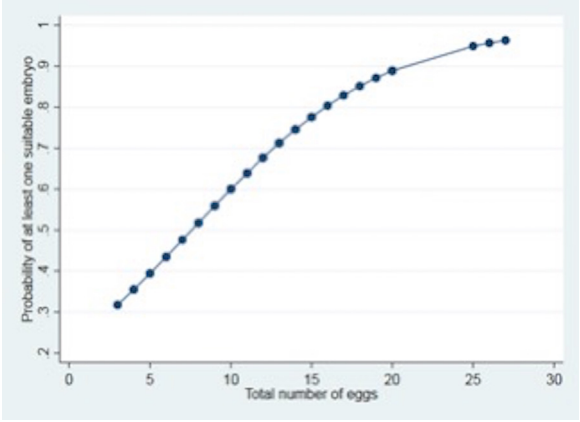

Fig. 8. Graph showing the probability of having at least one suitable embryo to transfer in patients undergoing PGT-SR against the total number of oocytes retrieved.

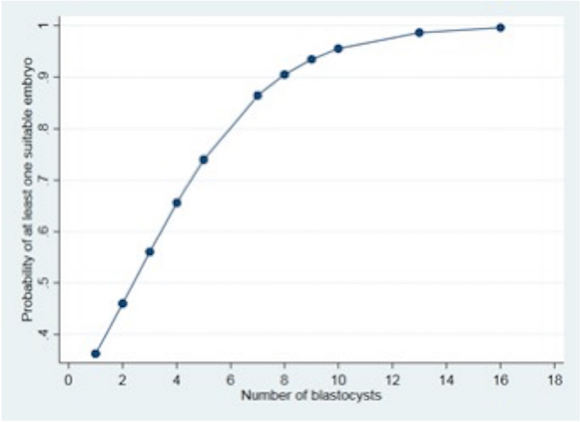

Fig. 9. Graph showing the relationship between the probability of having at least one suitable embryo to transfer in patients undergoing PGT-SR against the number of blastocysts biopsied.

effect of the number of oocytes on the number of suitable embryos to transfer was significant (p = 0.02, IRR 1.05;95%CI 1.01–1.10). The number of blastocysts biopsied was also a significant predictor of the odds of at least one suitable embryo to transfer (p = 0.007; OR 1.59; 95% CI 1.13–2.24) (Fig. 9).

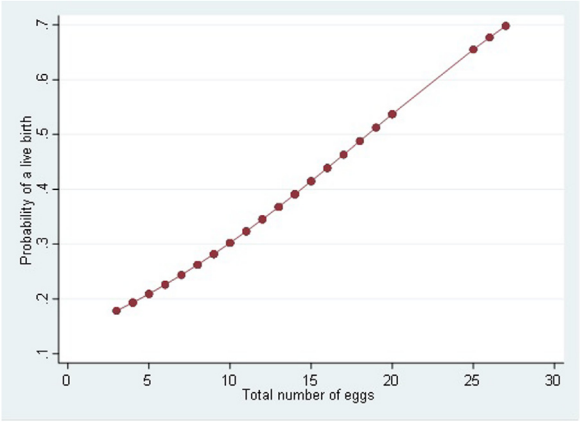

Fig. 10. Graph showing the probability of having a livebirth against the number of oocytes retrieved in patients undergoing PGT-SR.

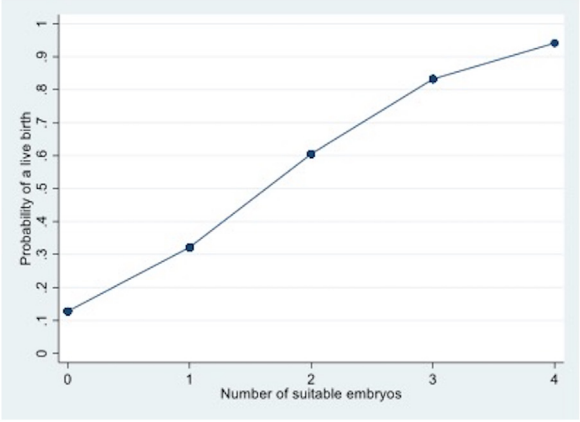

Fig. 11. Graph showing the relationship between the probability of a live birth and the number of suitable embryos in patients undergoing PGT-SR.

In contrast, the total number of oocytes collected was not significantly associated with the odds of a live birth (p = 0.09; OR 1.10; 95% CI 0.99–1.23) (Fig. 10). The number of blastocysts biopsied (p = 0.03; OR 1.22; 95% CI 1.02–1.47) and the number of suitable embryos to transfer had a significant effect on the odds of a live birth (p = 0.002; OR 3.23; 95% CI 1.54–6.80) (Fig. 11).

Conclusions

This study has demonstrated that the live birth following PGT-M and PGT-SR is significantly associated with the numbers of oocytes collected, blastocysts biopsied and suitable embryos to transfer. This is the first study which assesses these parameters in the context of PGT-M and PGT-SR outcome following blastocyst biopsy, vitrification and frozen embryo transfer.

The positive association between oocyte yield and live birth outcome in fresh and frozen IVF cycles has been well established [1,13]. Following analysis of >400, 0000 IVF cycles, Sunkara et al. (2011) demonstrated a strong association between the number of oocytes retrieved and live birth rate, where it increased with number of oocytes up to 15, plateaued between 15 and 20 and steadily declined beyond 20 oocytes [1]. Oocyte yield is also an important predictor for successful PGT outcome. In this study, there was a significant relationship between oocyte yield, number of blastocysts biopsied and suitable embryos to transfer in the whole group analysis. In this model, if age increases by one year, the number of blastocysts for biopsy and suitable embryos to transfer decreases by 2% and 6%, respectively. The area under the ROC curve indicated the number of oocytes collected was a good predictor for both number of blastocysts biopsied and suitable embryos to transfer. The number of oocytes retrieved was also significantly associated with the probability of a live birth. As the number of oocytes increased by one, there is a 6% increase in the odds of a live birth.

Similar to this study, Vandervorst et al. (1998) also demonstrated a positive correlation between number of oocytes retrieved and number of embryos biopsied [2]. Although embryo biopsy was carried out on day-3 and fresh transfer was undertaken, there was a significant correlation between the group where < 9 oocytes were retrieved and ≥ 9 oocytes with respect to number of embryos biopsied, number of embryos transferred and number of cycles resulting in embryo transfer.

In the current study, the number of blastocysts biopsied has a significant impact on the number of suitable embryos to transfer. For each additional blastocyst biopsied, there is a 61% increase in likelihood of having a suitable embryo to transfer with a predictive power of 0.8. Moreover, for each additional suitable embryo to transfer, the live birth increases by 38% with a ROC curve of 0.71. Thus, the number of blastocysts biopsied as well as the number of suitable embryos to transfer has a moderate-high predictive power on PGT outcome.

Further sub-analysis of couples embarking on PGT for single gene and chromosomal rearrangement also showed significant correlations between oocyte yield, number of blastocysts biopsied, number of suitable embryos to transfer and odds of a live birth. For each additional suitable embryo to transfer, there is a 1.28- and 3.23-fold increase in the odds of a live birth for single gene and chromosomal rearrangement, respectively.

The live birth rate following blastocyst biopsy, vitrification and frozen embryo transfer was 53% in our study, which is higher than that reported by ESHRE consortium XIV-XV of 25% [14]. The higher live birth rate can be attributed to the blastocyst biopsy in this study, which was only carried out in 4% of the PGT cycles reported to the ESHRE consortium. Furthermore, aneuploidy testing was also carried out in this study which showed that approximately a third of the embryos were aneuploid even when they were genetically suitable for transfer.

Some couples seeking PGT have diminished ovarian reserve and were counselled about the risk of poor response to stimulation and high risk of cancellation. For some couples, embryo batching was carried out to maximise egg yield, number of blastocysts to biopsy and eventually a suitable embryo to transfer. By extrapolating the results of this study, the probability of live birth can be determined from the number of oocytes collected and number of blastocysts biopsied. This can assist reproductive medicine specialists in counselling patients and enhances couple's ability to make fully informed decision about whether to continue with further embryo batching cycles or proceed with genetic analysis. In the whole group analysis, if 10 oocytes are retrieved, the live birth rate was approximately 40%. The probability of a live birth is ~65% when four suitable embryos are available to transfer and decreases to just under 50% when two suitable embryos are available to transfer.

Limitations of this study include its retrospective design and the nature of obtaining data from a single centre. We have demonstrated that a surrogate of a suitable embryo to transfer is egg yield and thus patients should be stimulated with a higher dose and/or in an aggressive step up protocol than for standard IVF patients in order to maximise egg and number of blastocysts to biopsy. Given that most PGT centres have adopted blastocyst biopsy and freeze protocols, a more aggressive stimulation approach can be adopted owing to the reduced risk of ovarian hyper stimulation syndrome associated with frozen cycles. We demonstrate herein a clear relationship between PGT outcome and ovarian response determined by the number of oocytes retrieved, blastocysts to biopsy and suitable embryos to transfer.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgment

The authors are grateful for the statistical input carried out by Dr A. Petrie for the manuscript.

References

[1] Sunkara S, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coormarsamy A. Association between the number of oocytes and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod 2011;201(26):1768–74.
[2] Vandervorst M, Liebaers L, Sermon K, Stassen C, De Vos A, Van de Velde H, et al. Successful preimplantation genetic diagnosis is related to the number of cumulus oocyte complexes. Hum Reprod 1998;13:3169–79.
[3] McArthur S, Leigh D, Marshall J, de Boer K, Jansen R. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril 2005;84:1628–36.
[4] Chang L, Huang C, Tsai Y, Hung C, Fang M, Lin Y, et al. Blastocyst biopsy and vitrification are effective for preimplantation genetic diagnosis of monogenic diseases. Hum Reprod 2013;28:1435–44.
[5] Scott R, Upham K, Forman E, Zhao T, Treff N. Cleavage stage biopsy significantly impairs human embryonic implantation potential while blastocysts biopsy does not: a randomized and paired clinical trial. Fertil Steril 2013;100:624–30.
[6] Chen Z, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med 2016;375:523–33.
[7] Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of fresh versus frozen embryos in ovulatory women. N Engl J Med 2018;37:126–31.
[8] Natsan S, Bladon A, Coskun S, Qubbaj W, Prates R, Munne S, et al. Genome-wide karyomapping accurately identifies the inheritance of single gene defects in human preimplantation embryos in vitro. Genet Med 2014;16:838–45.
[9] Konstantinidis M, Prates R, Neka Goodall N, Fischer J, Tecson V, Lemma T, et al. Live births following karyomapping of human blastocysts: experience from clinical application of the method. Reprod Biomed Online 2015;31:394–403.
[10] Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CG. Mol Hum Reprod 2008;14: 703–10..
[11] Fiorentino F, Spizzichino L, Bono S, Biricik A, Kokkali G, Rienzi L, et al. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. Hum Reprod 2011;26: 1925–35.
[12] Ben-Nagi J, Wells D, Doye K, Loutradi K, Exeter H, Drew E, et al. Karyomapping: a single centre's experience from application of methodology to ongoing pregnancy and live-birth rates. Reprod Biomed Online 2017;35:264–71.
[13] Vaughan D, Leung A, Resetkova N, Ruthazer R, Penzias A, Sakkas D, et al. How many oocytes are optimal to achieve multiple live births with one stimulation cycle? The one-and-done approach. Fertil Steril 2017;107:397–404.
[14] De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C, et al. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow up to October 2013. Hum Reprod 2017;201(32):1974–94.