Analysis of ten years of social oocyte cryopreservation: a research article

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Abstract

Objective: To assess the relationship between number of oocytes retrieved during social egg freezing (SEF) cycles with various clinical, biochemical and radiological markers; e.g. age, body mass index (BMI), baseline anti-Mullerian hormone (AMH), antral follicle count (AFC), Oestradiol level (E2) and total number of follicles [?]12mm at trigger. Main outcome measures: To describe the characteristics and outcomes of women who underwent SEF. Methods: A retrospective cohort of women embarking on SEF between 2008 and 2018 from a single London UK fertility clinic. Results: 483 stimulation cycles were undertaken in 373 women. The median age at freeze was 38 (26-47) years. The median numbers of oocytes retrieved per cycle was 8 (0-37), and total oocytes cryopreserved 8 (0-45) per woman. BMI, E2 level and number of follicles [?]12mm at trigger were all significant predictors of oocyte yield. Multivariate analysis confirmed no significant relationship between AFC or AMH, whilst on univariate analysis statistical significance was proven. 36 women returned to use their oocytes, with 41 autologous egg thaw cycles undertaken. 12 successful livebirths were achieved by 11 women. The overall livebirth rate was 26.8% per cycle. No livebirths occurred in women [?]40 years old and 82% of all livebirths were in women aged 36-39 at freeze. Conclusions: This study demonstrates clinical, biochemical and radiological markers can predict oocyte yield in SEF cycles. However, subsequent reproductive outcomes highlight women embarking upon SEF should be encouraged to do so before the age of 37 years, and no later than 40 to optimise successful livebirth.

Introduction

The development of oocyte vitrification, with success rates now similar to fresh cycles,[1] has enabled the opportunity to electively cryopreserve oocytes prior to the physiological decline in oocyte quality and quantity. Referred to herein as social egg freezing (SEF), it negates the age-associated deterioration in reproductive potential, but remains a controversial option owing to the multitude of ethical, legal, economic and obstetric related issues that it provokes.[2] Whilst some have little alternative option, such as single women approaching their late-thirties who desire biologically related children,[3] other indications include delaying childbearing for furthering education or to focus on career.[4]

Despite the theoretical benefit of preserving reproductive potential through SEF, it does not guarantee against involuntary childlessness.[5] Following SEF, if women return to use their cryopreserved oocytes; risk of loss during thawing or following unsuccessful embryo transfer may result in potential exhaustion of cryopreserved oocytes prior to achieving livebirth. Replenishment may subsequently be difficult in the same woman at a later age owing to the inevitable physiological depletion in ovarian reserve.[6] Therefore, at the

time of SEF, it is important to maximise the number of oocytes retrieved for cryopreservation to improve the probability of a successful livebirth.[7]

Various characteristics are associated with oocyte yield such as age at time of oocyte retrieval.[8] This encapsulates the very principle of SEF, and directly relates to the progressive depletion in primordial follicles and reduced neuroendocrine response to ovarian stimulation. This clinically manifests with poorer oocyte quantity and quality, as demonstrated by multivariate analysis to control for other confounding variables.[9] Contrarily, increased BMI is associated with reduction in yield.[10]

The most accurate markers of ovarian reserve considered for prediction of oocyte yield following controlled ovarian stimulation are antral follicle count (AFC) and anti-Mullerian hormone (AMH).[11] Endocrine markers including Oestradiol (E2), a measure of granulosa cell function has also been used.[12] Elevated levels of follicle stimulating hormone (FSH) on day 2-3 of a cycle is associated with a significant reduction in yield, when compared to women with lower FSH of the same age.[13] Radiologically, a follicular volume between 13-23mm/1-6mL at the time of ovulation trigger is also associated with optimal oocyte yields.[14]

The aim of this study was to assess the relationship between oocyte yield with clinical, biochemical and radiological markers including age, BMI, AMH, AFC, E2 and total number of follicles [?]12mm at trigger. A secondary aim was to report the reproductive outcomes following SEF in one of the largest reported cohorts in the UK so far.

Methods

Selection criteria

This retrospective cohort includes all women who approached the Centre for Reproductive and Genetic Health in London, UK for SEF over a ten-year period between 2008 and 2018.

Clinical Protocols

An initial assessment was carried out, which included a blood test for AMH (Roche Assay), FSH, E2 and ultrasound for AFC performed between days 1-4 of the menstrual cycle. The ovarian stimulation protocol was individualised depending on age, AFC, AMH and BMI. Ovarian stimulation using an antagonist protocol was commenced on the third day of the menstrual cycle. From day 6 onwards, the gonadotrophin dose was adjusted according to E2 levels and ultrasound evidence of stimulation. On day 7 of the cycle and/or once the leading follicle was [?]14 mm in diameter, Cetrorelix (Cetrotide) 0.25 mg (Merck Serono, Germany) was commenced. The choice of trigger was individualised after assessment of age, baseline AFC and AMH, number of follicles and E2 levels on the day of trigger. Women considered low responders were given either Pregnynl a human chorionic gonadotrophin (hCG) trigger, or dual trigger with Pregnyl 1,500 IU (Organon, Netherlands) and Suprefact 1ml (Sanofi-Aventis, Germany), a gonadotrophin releasing hormone analogue (GnRH). Women considered normal or high responders were given Suprefact only. Oocyte maturation was triggered once the mean diameter of [?]3 follicles [?]18mm. In the event women were at high risk of ovarian hyperstimulation syndrome (OHSS), Suprefact 1ml (Sanofi-Aventis, Germany) was administered and LH level was measured 8-12 hours later. Oocyte retrieval was scheduled 37 hours after ovulation trigger. Denudation was carried out 39 hours post the trigger injection and metaphase II oocytes were vitrified immediately after.

All thaw cycles included within our study refer to the thawing of oocytes which were frozen initially at CRGH, undergoing the vitrification method. Vitrification at room temperature (24-26°C) took place in two steps. Firstly, once oocytes were denuded they were moved for 12-15 minutes within a solution containing 7.5% Dimethyl Sulfoxide-D6 (DMSO) and 7.5% Ethylene Glycerol (EG). Oocytes achieving full recovery to normal size were then transferred to a vitrification solution consisting of 15% DMSO and EG plus 0.5-m sucrose for 60 seconds. Secondly, oocytes were then transferred via Cryotop straws at fast rate into liquid nitrogen for storage, within a minimum volume of vitrification solution. Six welled dishes adjacent to three welled plates from Cryotech were used containing solutions warmed at 37° with various concentrations of sucrose (1.0. 0.5 and 0.0m). The vitrified oocytes within the Cryotop straws were then removed from storage and moved to liquid nitrogen, within 0.7ml 1.0 M of sucrose, followed by 3-5 minutes within 50 µl of varying

decreasing sucrose concentrations at room temperature. Oocytes with full recovery were then transferred for 2 hours into culture before undergoing intracytoplasmic sperm injection (ICSI).

The oocytes were assessed 16-18 h post ICSI for the presence of pronuclei and were separated according to the number of pronuclei present. Oocytes displaying abnormal pronuclei numbers (zero, one and more than three) were either discarded or kept in a separate dish of cleavage medium (Vitrolife). Oocytes displaying 2 pronuclei were either moved to a fresh dish of cleavage medium (Vitrolife) while the two-step culture system was in effect, or single-step culture media (Sage/Origio). The embryos were either moved to Blastocyst medium (Vitrolife) or a fresh dish of single-step media (Sage/Origio) on day 3 in order to be cultured to the blastocyst stage, until day 5 or 6 post insemination. Any embryos that successfully formed a blastocyst on day 5 or 6 of culture (exhibiting the presence of clear inner cell mass and trophectoderm cell lines) were selected for vitrification.

Blastocyst vitrification took place at 37°C. Multiple 5-well Nunc dishes were used to warm 1 x 0.5ml wells of V1 per blastocyst, 1 x 0.5ml well of V2 and 1 x 1ml well of V3 media solutions (Blastocyst Vitrification Kit, Sydney IVF) to 37°C a minimum of 1 hour prior to use. Blastocysts were isolated into micro-drops of warm Hepes media under oil, assessed at 200x magnification and graded as per the CRGH in-house grading criteria (adapted Cornell). Blastocoelic cavity collapse was then initiated by a single laser shot to the junction of two outermost trophectodermal cells, before the blastocysts were transferred to individual wells of the warmed V1 media (up to 4 blastocysts per dish). Vitrification was conducted by moving a single blastocyst from V1 to V2 media for 2 minutes followed by thorough washing through 3 drops of V3 media for 30-35 seconds. The blastocyst was then loaded rapidly onto a Cryolock with minimal volume of vitrification solution and plunged into liquid nitrogen before placing a protective cap over the loading strip. Once blastocysts were frozen, women embarked on a single elective embryo transfer. A medicated frozen embryo transfer was performed.[15] All patients were advised to perform a urinary pregnancy test 16 days later.

Details of Ethics Approval

Ethics approval was not necessary for this retrospective study as vitrification of oocytes is a validated technique that should no longer be considered experimental.

Statistical analyses

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as median ± range. Multivariate Poisson regression was used to evaluate the effect of the aforementioned variables with the number of oocytes retrieved. Mann Whitney U test was used to determine a relationship between the variables and their effect on OHSS. The Kruskal Wallis test and Mann Whitney U tests were used to determine significance between age groups and probability of livebirth based upon their age and number of oocytes retrieved. Chi squared testing was utilised to determine statistical significance in those who returned to use their oocytes, to assess between age groups and achievement of livebirth. Statistical significance was set at $p<0.05$.

Results

483 stimulation cycles were undertaken in 373 women. The median age (SD) at freeze was 38.3 (±3.5). The median number of cycles per woman was 1 (range 1-8). The median number of oocytes retrieved per cycle was 8 (0-37), whilst the number of metaphase II oocytes was 6 (0-28). The median BMI was 22.5 (17.1-37) kg/m. The median baseline AMH was 9.9 (0.17-56.6) pmol/l and the AFC was 12 (2-49). At ovulation trigger, the median E2 level was 7.01 (0.25-30.26) per 1000 pmol/l and the total number of follicles [?]12mm was 9 (1-50). (**Table 1)**

Univariable Poisson regression was used to assess the effect of each variable on oocyte yield (**Table 2)** . This identified that age, baseline AFC, AMH, E2, and follicle count [?]12mm on the day of trigger were all significant predictors of oocyte yield ($p<0.001$). Multivariable Poisson regression subsequently demonstrated that BMI (Incidence rate ratio; IRR; 1.02; 95% CI 1.00 -1.03; 95% p=0.04), E2 level per 1000 pmol/l increase (IRR 1.05; 95% CI 1.04 -1.07; $p<0.001$) and the number of follicles [?]12mm at trigger (IRR 1.02; 95% CI

1.01-1.03; p<0.001) were all significant predictors of oocyte yield (**Table 3)** . However, there was no significant relationship between AFC (IRR=1.00; 95% CI 0.99-1.02 p=0.43) or AMH (IRR 1.00; 95% CI 0.99-1.01; p=0.71) with oocyte yield. An increase in age by one-year resulted in a 4% reduction in yield after adjusting for all other variables (IRR 0.96; 95% CI 0.94-0.98; p<0.001).

There were nine cases in total (2.4%) of OHSS; all of which classified as mild in severity as per the Royal college of Obstetrician and Gynaecologist classification.[16] In regards to mode of trigger, there were four cases of hCG, four Suprefact and one case of dual trigger. There were no associated hospital admissions or significant adverse events. Analysis using the Mann Whitney U test identified that age, total AFC, number of follicles more than 12mm on the day of trigger and FSH were all significant predictors of OHSS (p<0.05). BMI, AMH and E2 levels however were not significant predictors of OHSS (**Table 4).**

Thirty-six women subsequently underwent 41 oocyte thaw cycles. The mean age at thaw was 42 (+-4.1). The mean number of years between cryopreservation and thaw was 3.7 years. Almost two thirds (n=23) of women used partners sperm, whereas 36% (n=13) used donor sperm. Of those who used donor sperm, the majority did so because they were single (n=9, 69%).

The percentage of oocytes which survived thaw was 81, 76%, 67.5% in women aged [?]35, 36-39 and [?]40 years respectively. The fertilization rate of the frozen thawed oocyte was 53%, 68% and 58% in the respective age groups. There were 37 embryo transfers (ET). Almost half of the embryo transfers took place at the blastocyst stage 48.6%, (n=18). Of those cycles with all embryos cultured to the blastocyst stage, the average number of blastocysts formed were 55.6% (10/18), 45.5% (40/88) and 50% (9/18) in the respective age groups.

Following embryo transfer, 13 women had confirmed clinical pregnancies resulting in a clinical pregnancy rate (fetal heart visualised on ultrasound) of 31.7% per thaw cycle. 12 livebirths were subsequently achieved by 11 women (one twins), attributing a livebirth rate of 26.8% (11/41) per cycle and 29.7% per ET (11/37). The remaining two women had missed miscarriages, having both first cryopreserved their oocytes above 40 years old.

In women aged [?]35 years, two livebirths from six egg thaw cycles were achieved, resulting in a livebirth rate per egg thaw cycle of 33%. In those aged 36-39, 24 cycles led to 9 livebirths resulting in a livebirth rate of 37.5% per egg thaw cycle. In those aged 40 years and over, all 11 cycles were unsuccessful, resulting in a livebirth rate of 0% as demonstrated in **Figure 1.** The average age at cryopreservation of those who achieved a livebirth was 36.4, whereas those who did not was 39.0 (p=0.07).

Discussion

Interpretation

This study demonstrates herein that markers such as E2, BMI and the number of follicles [?]12mm at trigger are all independent significant predictors of oocyte yield in women undergoing SEF. However, whilst AFC and AMH were significant predictors of oocyte yield utilising univariate analysis, the relationship was not significant when controlling for other potential confounders. Whilst there are few previous studies assessing these relationships in the context of SEF, comparisons can be made from data from cycles performed for other indications, with consideration for the differences in population characteristics.

AMH is considered a reliable predictor of oocyte yield.[17] However, it is well known that AMH levels are strongly correlated with age.[18] This study demonstrates herein that AMH was a statistically significant predictor of yield utilising univariate analysis, but when controlling for other variables using multivariate analysis, no significant relationship was identified. We propose therefore, that when assessing oocyte yield controlling for confounders such as age; such interrelationships may nullify the impact of AMH.

It is important to emphasise that women undergoing SEF are young and fertile, unlike the vast majority of women undergoing IVF. AMH is often used as a surrogate marker of ovarian reserve in women undergoing ART, and therefore much of the evidence is limited to women with diminished ovarian reserve.[19] Causes

of subfertility include previous ovarian surgery or following gonadotoxic treatment with chemotherapy or radiotherapy, resulting in diminished AMH regardless of age.[20] As such it is unsurprising that data in infertile populations; AMH has been shown to be an independent significant predictor of oocyte yield.[21] However, as demonstrated herein, in women who undergo SEF, age remained an independent significant predictor even when controlling for other variables, highlighting it as a more accurate predictor of oocyte yield than AMH in this population.

BMI is also associated with oocyte yield.[22] However, whereas it was previously identified that oocyte quantity was lower in obese women, compared with those with a normal BMI,[23]the findings demonstrated herein contrarily show that oocyte yield increased by 2% per kg/m^2 increase of BMI. The majority of women in our cohort had a normal BMI 20.0-24.9 (n=111), less were categorised as being underweight (BMI <20.0, n=34) and very few were obese (BMI >30.0, n= 9). In contrast to previous literature, the mean number of oocytes collected in the obese group was higher than the underweight group, although not to a significant extent (9.6 vs 8.5). This is further evidence that in the context of SEF, patient demographics are dissimilar to the standard IVF population. Obese infertile women may have associated subfertility related to their body habitus, such as those with polycystic ovarian syndrome; however overweight fertile women may not have a discernible reduction in oocyte yield. This is exemplified by previous studies where obese women contributed to 8.9% of the total cohort, compared to only 2.9% in our own cohort.[24] This reinforces further that much of the evidence on prediction of oocyte yield deduced from IVF cycles in infertile women may not be extrapolatable to SEF populations.

At the time of writing 9.7% of the women who underwent SEF had returned to use their oocytes, a mean of 3.7 years after cryopreservation. A previous study reporting outcomes from 1382 women who underwent SEF at a mean age of 37.7 years showed that 8.7% (n=120) women returned to use their oocytes after a short follow up time of 2.2 years.[25] Another study, using a smaller sample of 254 women in Sweden, reported that 15% (n=38) returned to use their oocytes after a longer follow up period, returning after a mean of 4 years,[26] whilst another survey reported just 6% of 96 women returned to use their oocytes, 2.8 years after undergoing SEF.[27] Current utilisation rates appear low as some women, particularly those less than 35 years of age may subsequently spontaneously conceive whilst their oocytes are cryopreserved.

The clinical pregnancy rate demonstrated in our cohort of women was 31.7% with a median age of 43 years old. This is comparable to the average pregnancy rates reported by the Human Fertilisation and Embryology Authority (HFEA) in women aged between 35-37 years.[28] Such findings epitomises the reproductive potential of SEF and provides further clinical correlation of the concept. Just under a third (30.5%) of women who returned to use their stored oocytes attained a livebirth. This is favourable compared to a recent study analysing 10 years of data from two UK clinics who had a success rate, pertaining to livebirths or ongoing pregnancies of 17.5%.[29] In that study, which had a similar mean age at cryopreservation of 37 years, successful outcomes including a livebirth or ongoing pregnancy were achieved in 20-23% of women aged <40. In the data presented herein, the success rate in those who cryopreserved oocytes <40 years was higher, at 37% (n=11/30). In the Swedish study that published outcomes of 38 women after SEF, the cumulative live birth rate was 63%, 26% and 0% in women of ages 36-37, 38-39 and [?]40 years at vitrification respectively.[26] When stratifying using similar age groups in the cohort presented herein, this data presents findings, with livebirth rates in women who underwent SEF [?]35 years of 33% (n=6), 37.5% aged 36-39 (n=24), and 0% [?]40 years (n=11).

In those women who underwent SEF over the age of 40 years old, no women who returned to thaw their oocytes had a successful outcome, and of those, almost three quarters (72.7%) had exhausted their oocyte store. This proportion is more than double that reported following assessment of outcomes from the aforementioned Swedish study, where in the 11 women who underwent SEF over the age of 40, none achieved a livebirth, and four (36%) had exhausted their oocyte supply.[26] The previously discussed UK study across two centres fared slightly better, with 7% of those aged 40-42 having a successful outcome.[29] Whilst the overall numbers remain small, it is clear undergoing SEF aged 40 or over is unlikely to be successful. Not only are the success rates low, but the risk of miscarriage remains elevated, exemplified by the fact the two

women who suffered miscarriages in this study were both aged >40. This is reinforced by data that has identified the risk of miscarriage in women aged 40-44 is 51%.[30]

This reaffirms that if women choose to undergo SEF, it is likely to offer most benefit to do so by the age of 36. However, caution should be taken advising women to cryopreserve 'too' early. Each case should be individually considered, with appropriate contemplation of the physiological, obstetric, legal and economic considerations.[31] Although storing oocytes earlier optimises oocyte quality and enhances success rates, the likelihood of spontaneous conception in those additional reproductive years is greater, which would potentially have resulted in unnecessary physical risk and substantial financial burden, in addition to the expense of longer oocyte storage. Moreover, in the context of UK legislation, current HFEA regulations permit the storage of oocytes for a maximum period of 10 years.[32] Therefore, storing oocytes earlier to optimise future success may be counterproductive, necessitating the need to prematurely use a sperm donor, to prevent the oocytes being discarded. However, following concern regarding the appropriateness of this legal time frame,[4,33] it is possible this legal chronological restriction may be alleviated in the future.

The findings of this study add substantially to published literature to identify factors that impact success following SEF. The associated enhanced ability to counsel women regarding their probability of oocyte yield, and subsequent livebirth is important as some women may not wish to continue with cryopreservation if there is a suboptimal chance of success. Whilst speculative, if women in this cohort were aware they had a very low chance of success, they may have undergone further cycles to optimise their future chances, or indeed if are 40 and above, not embark upon treatment in the first place. Other studies have shown that women are willing to undergo two or more cycles in order to retrieve sufficient numbers of oocytes for storage.[34]Therefore, it is essential women have the most accurate individualised estimate of their likelihood of future success, in order to make informed choices regarding number of cycles and facilitate the management of future expectations. However, it is paramount that no matter how high the predicted percentage chance of achieving a livebirth, women are made aware that the likelihood of a livebirth cannot be guaranteed. This is important given evidence has suggested women who overestimate expectancy of livebirth, or have few oocytes to preserve often regret freezing, resulting in subsequent emotional and psychological sequelue.[35]

Limitations

Limitations of this study include that all participants were from a single centre, and as such, there is potential for bias. Moreover, the decision to give either GnRH antagonist or dual trigger were made on an individual basis and based on clinical judgment, including consideration of previous response to stimulation along with baseline AMH and AFC and E2 levels on the day of trigger. The lack of strict criteria introduces the potential for bias in this analysis. All of the above could be minimised by the use of a multi-centre randomised powered study.

Conclusion

This study highlights that age, BMI, E2, and number of follicles [?]12mm at trigger can predict oocyte yield in SEF cycles. As such, individualising SEF cases with consideration of these markers, should be undertaken to optimise oocyte yield. AMH and AFC are however not independently significantly associated with oocyte yield. Additionally, this is the first study to establish an incidence of OHSS in SEF cycles and establish a significant relationship between age, total AFC, number of follicles [?]12mm and FSH as predictors of OHSS. Moreover, the reproductive outcomes presented highlight that undertaking SEF over the age of 40 years is suboptimal and associated with a low chance of success. Whilst there was no significant difference in livebirth rates in women [?]35 years and aged 36-39, it is advisable that SEF should be undertaken at or prior to the age 36 to optimise outcomes. This data has significant implications for clinical practice, and can be used to individualise care, enhance counselling and manage expectations in women undergoing SEF.

Disclosure of interests

The authors of this manuscript have no conflicts of interest to declare.

Contribution of authorship

LSK and BPJ instigated and wrote the manuscript. CH, RO and JG were involved in the data collection. AP carried out the statistical analysis. SS and PS were involved in the critique of the manuscript. JBN was involved in the conception and final critique of the manuscript. All authors reviewed the final draft.

Ethics Approval

Ethics approval was not necessary for this retrospective study as vitrification of oocytes is a validated technique that should no longer be considered experimental.

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Table 1 Demographic data and controlled ovarian stimulation variables

	Median (range)
Age (years)	38.3 (26-47)
BMI (Kg/m^2)	22.5 (17.1-36.6)
FSH (IU/l)	7.2 (1.1-13.7)
AMH (pmol/l)	9.9 (0.17-56.6)
Baseline antral follicle count	12 (2-49)
Number of days of stimulation	9 (4-14)
Total dose of stimulation IU	3562.5 (700-13875)
Antral follicles > 12mm on day of trigger	9 (1-50)
Oestradiol levels on day of trigger (per 1000 pmol/l)	7.01 (0.25-30.26)
Number of oocytes retrieved	8 (0-37)
Number of metaphase II	6 (0-28)
Number of Metaphase I	0 (0-8)
Number of Germinal Vesicles	0 (0-19)

	IRR	95% CI	p-value
Age (years)	0.92	0.91-0.93	<0.001
BMI (kg/m^2)	0.99	0.97-0.99	0.019
AFC	1.39	1.03-1.04	<0.001
AMH (pmol/l)	1.02	1.03-1.02	<0.001
Oestradiol on day of trigger (per 1000 pmol/l)	1.08	1.07-1.09	<0.001
Number of follicles >12 mm on day of trigger	1.04	1.04-1.04	<0.001

Table 2 The incidence rate ratio (IRR), confidence interval (CI) and p-values to assess the effect of each variable on oocyte yield (univariate analysis)

Table 3 The incidence rate ratio (IRR), confidence interval (CI) and p-values to assess the effect of each variable on oocyte yield (multivariate analysis)

	IRR	95% CI	p-value

	IRR	95% CI	p-value
Age (years)	0.96	0.94-0.98	<0.001
BMI (kg/m^2)	1.02	1.00-1.03	0.04
AFC	1.00	0.99-1.02	0.43
AMH (pmol/l)	1.00	0.99-1.01	0.71
Oestradiol on day of trigger (per 1000 pmol/l)	1.05	1.04-1.07	<0.001
Number of follicles >12 mm on day of trigger	1.02	1.01-1.03	<0.001

Table 4 Mann Whitney U, interquartile range (IQR) and p-values to assess the effect of each variable on oocyte yield on OHSS and Non-OHSS groups

Ovarian reserve marker	**OHSS median (IQR)**	**Non OHSS median (IQR)**	**p value**
BMI (Kg/m^2)	20.8 (5.75)	21.8 (4.0)	0.347
Age (years)	37.0 (3.5)	38.0 (4.0)	**0.019***
Total AFC	16.5 (22.0)	12.0 (8.0)	**0.027***
AMH (pmol/l)	25.8 (25.5)	9.6 (10.9)	0.055
No follicles >12mm	15.0 (11.0)	9.0 (7.0)	**0.001***
FSH (IU/l)	6.1 (2.5)	7.3 (3.9)	**0.006***
Oestradiol (per 1000 pmol/l)	123.0 (154.5)	147.8 (137.0)	0.361

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`Figure 1* No thaw cycles per age group.pdf` available at https://authorea.com/users/364849/articles/485719-analysis-of-ten-years-of-social-oocyte-cryopreservation-a-research-article