Human Reproduction, Vol.35, No.5, pp. 1082–1089, 2020
Advance Access Publication on April 29, 2020 doi:10.1093/humrep/deaa028

human reproduction

ORIGINAL ARTICLE *Infertility*

No effect of ovarian stimulation and oocyte yield on euploidy and live birth rates: an analysis of 12 298 trophectoderm biopsies

M. Irani[1,*], C. Canon[2], A. Robles[2], B. Maddy[3], V. Gunnala[1], X. Qin[1], C. Zhang[1], K. Xu[1], and Z. Rosenwaks[1]

[1]The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY 10021, USA, [2]Obstetrics and Gynecology, New York Presbyterian/Weill Cornell Medicine, New York, NY 10021, USA, [3]Weill Cornell Medicine, New York, NY 10021, USA

*Correspondence address. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine 1305 York Avenue, 6th floor, New York, NY 10021, USA. Tel: 1-646-962-3413; Fax: 646-962-0392; E-mail: mohamad.irani@hotmail.com

Submitted on December 21, 2019; resubmitted on January 22, 2020; editorial decision on February 9, 2020

STUDY QUESTION: Does ovarian stimulation affect embryo euploidy rates or live birth rates (LBRs) after transfer of euploid embryos?

SUMMARY ANSWER Euploidy rates and LBRs after transfer of euploid embryos are not significantly influenced by gonadotropin dosage, duration of ovarian stimulation, estradiol level, follicle size at ovulation trigger or number of oocytes retrieved, regardless of a woman's age.

WHAT IS KNOWN ALREADY: Aneuploidy rates increase steadily with age, reaching >80% in women >42 years old. The goal of ovarian stimulation is to overcome this high aneuploidy rate through the recruitment of several follicles, which increases the likelihood of obtaining a euploid embryo that results in a healthy conceptus. However, several studies have suggested that a high response to stimulation might be embryotoxic and/or increase aneuploidy rates by enhancing abnormal segregation of chromosomes during meiosis. Furthermore, a recent study demonstrated a remarkable difference in euploidy rates, ranging from 39.5 to 82.5%, among young oocyte donors in 42 fertility centres, potentially suggesting an iatrogenic etiology resulting from different stimulation methods.

STUDY DESIGN, SIZE, DURATION: This is a retrospective cohort study that included 2230 *in vitro* fertilisation (IVF) with preimplantation genetic testing for aneuploidy (PGT-A) cycles and 930 frozen-thawed single euploid embryo transfer (FET) cycles, performed in our centre between 2013 and 2017.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 12 298 embryos were analysed for ploidy status. Women were divided into five age groups (<35, 35–37, 38–40, 41–42 and >42 years old). Outcomes were compared between different durations of stimulation (<10, 10–12 and ≥13 days), total gonadotropin dosages (<4000, 4000–6000 and >6000 IU), numbers of oocytes retrieved (<10, 10–19 and ≥20 oocytes), peak estradiol levels (<2000, 2000–3000 and >3000 pg/mL), and sizes of the largest follicle on the day of trigger (<20 and ≥20 mm).

MAIN RESULTS AND THE ROLE OF CHANCE: Within the same age group, both euploidy rates and LBRs were comparable between cycles regardless of their differences in total gonadotropin dosage, duration of stimulation, number of oocytes harvested, size of the largest follicles or peak estradiol levels. In the youngest group, (<35 years, $n = 3469$ embryos), euploidy rates were comparable between cycles with various total gonadotropin dosages (55.6% for <4000 IU, 52.9% for 4000–6000 IU and 62.3% for >6000 IU; $P = 0.3$), durations of stimulation (54.4% for <10 days, 55.2% for 10–12 days and 60.9% for >12 days; $P = 0.2$), number of oocytes harvested (59.4% for <10 oocytes, 55.2% for 10–19 oocytes and 53.4% for ≥20 oocytes; $P = 0.2$), peak estradiol levels (55.7% for E2 < 2000 pg/mL, 55.4% for E2 2000–3000 pg/mL and 54.8% for E2 > 3000 pg/mL; $P = 0.9$) and sizes of the largest follicle (55.6% for follicles <20 mm and 55.1% for follicles ≥20 mm; $P = 0.8$). Similarly, in the oldest group (>42 years, $n = 1157$ embryos), euploidy rates ranged from 8.7% for gonadotropins <4000 IU to 5.1% for gonadotropins >6000 IU ($P = 0.3$), from 10.8% for <10 days of stimulation to 8.5% for >12 days of stimulation ($P = 0.3$), from 7.3% for <10 oocytes to 7.4% for ≥20 oocytes ($P = 0.4$), from 8.8% for E2 < 2000 pg/mL to 7.5% for E2 > 3000 pg/mL ($P = 0.8$) and from 8.2% for the largest follicle <20 mm to 8.9% for ≥20 mm ($P = 0.7$). LBRs after single FET were also comparable between these groups.

LIMITATIONS, REASONS FOR CAUTION: Although this large study (2230 IVF/PGT-A cycles, 12 298 embryos and 930 single FET cycles) demonstrates the safety of ovarian stimulation in terms of aneuploidy and implantation potential of euploid embryos, a multi-centre study may help to prove the generalisability of our single-centre data.

WIDER IMPLICATIONS OF THE FINDINGS These findings reassure providers and patients that gonadotropin dosage, duration of ovarian stimulation, estradiol level, follicle size at ovulation trigger and number of oocytes retrieved, within certain ranges, do not appear to significantly influence euploidy rates or LBRs, regardless of the woman's age.

STUDY FUNDING/COMPETING INTEREST(S): No external funding was received and there are no competing interests to declare.

TRIAL REGISTRATION NUMBER: N/A

Key words: ovarian stimulation / oocyte yield / euploidy rate / live birth rate / preimplantation genetic testing for aneuploidy

Introduction

Women face a gradual decline in fertility after reaching age 35, which is reflected by the lower fecundability and higher miscarriage rates seen in older women (Leridon 2004; Steiner and Jukic 2016). This trend has been attributed to the steep increase in aneuploidy rates in women older than 34 years old (y.o.) (Franasiak *et al*. 2014). Hence, the principal goal of ovarian stimulation is to overcome these high aneuploidy rates and increase the likelihood of obtaining a euploid embryo (Sunkara *et al*. 2011; Steward *et al*. 2014). However, high oocyte yield is associated with increased risks of complications, including intra-abdominal bleeding, ovarian torsion and ovarian hyperstimulation syndrome (OHSS) (Bodri *et al*. 2008). OHSS, which is the most serious and potentially life-threatening complication, has been largely mitigated by the administration of GnRH agonist to trigger final oocyte maturation followed by the cryopreservation of all embryos in women with high ovarian response (Engmann *et al*. 2008).

Several studies have suggested that a high response to stimulation might be embryotoxic and/or increase aneuploidy rates by enhancing abnormal segregation of chromosomes during meiosis (Vogel and Spielmann 1992; Valbuena *et al*. 2001; Van der Auwera and D'Hooghe 2001; Lee *et al*. 2005; Roberts *et al*. 2005; Baart *et al*. 2007). Animal studies have indicated that superovulation delays embryonic and foetal development, and it may accelerate nuclear maturation and affect chromosome congression during prometaphase and metaphase, thus causing a higher risk of aneuploidies (Vogel and Spielmann 1992; Van der Auwera and D'Hooghe 2001; Lee *et al*. 2005; Roberts *et al*. 2005). Furthermore, a small study has suggested that stimulation with high-dose exogenous gonadotropins might lead to higher aneuploidy rates compared to mild stimulation (Baart *et al*. 2007). A recent small retrospective study also showed higher aneuploidy rates in women undergoing stimulation with higher gonadotropin dosages (Sachdeva *et al*. 2018). In addition, a high level of estradiol (E2) has been proposed to be embryotoxic, with a progressive reduction in embryonic adhesion occurring after exposure to gradually higher E2 levels (Valbuena *et al*. 2001). Moreover, a recent study demonstrated a remarkable difference in euploidy rates, ranging from 39.5 to 82.5%, among young oocyte donors in 42 fertility centres, potentially suggesting an iatrogenic etiology resulting from different stimulation methods (Munne *et al*. 2017).

Determining the effect of exogenous gonadotropins on embryo ploidy in women undergoing ovarian stimulation is critical for the selection of the best stimulation protocols. Therefore, this large study was conducted to identify whether the duration of stimulation, total gonadotropin dosage, number of oocytes retrieved, peak E2 level or follicular size on the day of trigger affect embryo aneuploidy rates or live birth rates (LBRs) of euploid embryos transferred in subsequent single frozen-thawed embryo transfer cycles (FET).

Materials and Methods

Cycle selection

The Weill Cornell Medicine Institutional Review Board approved this retrospective cohort study. All IVF cycles in which embryos were biopsied for preimplantation genetic testing for aneuploidy (PGT-A) at the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine between January 2013 and December 2017 were included.

Clinical protocols

Ovarian stimulation, trigger of final oocyte maturation, oocyte retrieval, fertilisation, embryo culture and transfer were carried out according to our standard protocols (Huang and Rosenwaks 2014). The stimulation protocol was selected based on patient age, weight, evaluation of ovarian reserve and response to previous stimulation. The patients received daily exogenous gonadotropins (Gonal-F, EMD-Serono Inc.; Follistim, Merck, Kenilworth, NJ, USA; and Menopur, Ferring Pharmaceuticals Inc., Parsippany, NJ, USA). The response to stimulation was assessed by measuring serum E2 levels and performing transvaginal ultrasounds. GnRH antagonist (Ganirelix acetate, Merck, Kenilworth, NJ, USA; or Cetrotide, EMD-Serono Inc., Rockland, MA, USA) or GnRH agonist (leuprolide acetate, Abbott Laboratories, Chicago, IL, USA) was used for pituitary suppression. Human chorionic gonadotropin (Novarel, Ferring Pharmaceuticals Inc., Parsippany, NJ, USA) and/or GnRH agonist (leuprolide acetate, Abbott Laboratories, Chicago, IL, USA) were used to trigger the final oocyte maturation. Ultrasound-guided oocyte retrieval was performed 35–37 h after the trigger.

Blastocysts were cryopreserved after biopsy, and the euploid embryos were transferred in FET cycles. In general, women with regular menstrual cycles underwent natural FET cycles in which they were monitored during the last few days of the follicular phase to detect the LH surge and ensure adequate endometrial development. The transfer was performed 5 days after the LH surge. Vaginal progesterone supplementation (Endometrin, Ferring Pharmaceuticals Inc., Parsippany, NJ, USA) was administered after embryo transfer to some women based on physician discretion. Alternatively, patients underwent programmed FET cycles in which E2 patches were applied for ~2 weeks before

Table I Demographic and embryo ploidy data for women who underwent IVF/PGT-A cycles.

	Age (years)					
Parameters	<35 ($n=497$)	35–37 ($n=440$)	38–40 ($n=604$)	41–42 ($n=389$)	>42 ($n=300$)	*P* value
Age (years)	31.6 ± 2.2	36.0 ± 0.8	39.1 ± 0.8	41.4 ± 0.5	43.7 ± 0.9	<0.001
BMI (kg/m^2)	23.2 ± 4.3	22.7 ± 3.7	23.7 ± 4.2	23.4 ± 4.0	24.1 ± 4.5	NS
Parity	0.4 ± 0.7	0.5 ± 0.7	0.4 ± 0.7	0.4 ± 0.7	0.5 ± 0.8	NS
# embryos biopsied	6.9 ± 4.2	6.1 ± 3.8	5.2 ± 3.3	4.6 ± 3.4	3.8 ± 3.1	<0.001
# euploid embryos	3.8 ± 2.9	2.8 ± 2.3	1.7 ± 1.6	0.8 ± 1.1	0.3 ± 0.7	<0.001
Euploidy rates (%)	55.4	44.8	32.4	18.2	8.5	<0.001
Cycles with no euploid embryos (%)	5.2	12.5	24.8	48.8	74.0	<0.001

This table compares the demographics, number of embryos biopsied and the ploidy data between the five age groups. BMI: body mass index. NS: not significant.

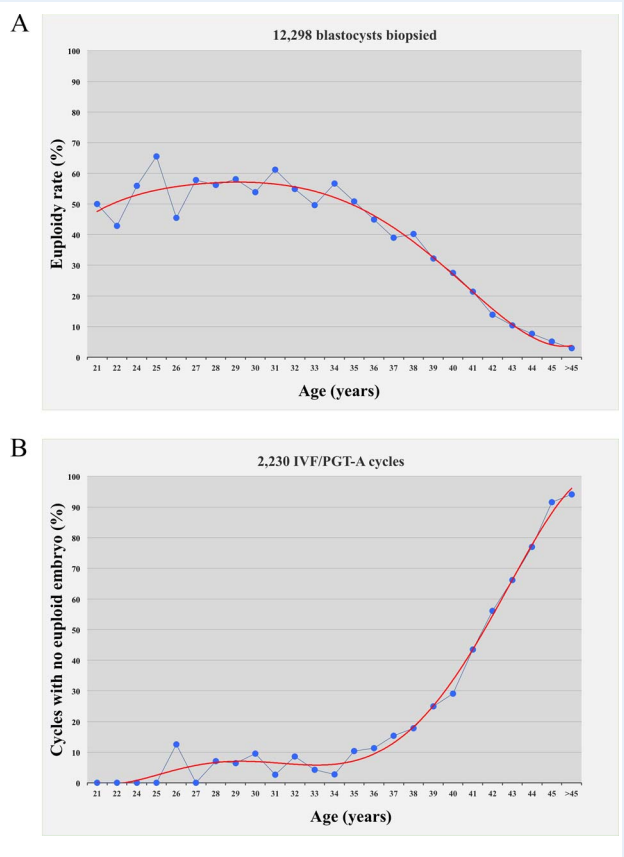

Figure 1 Embryo euploidy according to the woman's age. **A**: The association between women's age and euploidy rates. Euploidy rates are highest in young women and start to gradually decline in women older than 35 years of age. The fifth degree polynomial regression line delineates the changes in euploidy rates as women get older. **B**: The prevalence of IVF cycles in which no euploid embryo is obtained, relative to the woman's age.

starting intramuscular progesterone when the endometrial thickness reached ≥7 mm. The transfer was performed 6 days after starting progesterone.

Laboratory protocols

Embryos were cultured in sequential media using the EmbryoScope (Vitrolife) time-lapse system. Embryos were biopsied on Day 5 or 6 based on the time of blastulation. After immobilising the embryo with a holding pipette, the zona pellucida was perforated by laser pulses (ZILOS-tk Laser), and a biopsy pipette of 20-µm internal diameter was used to aspirate three to seven cells. The specimens were washed with a wash buffer and placed in 0.2-mL PCR tubes including 2 µL lysis buffer. The Weill Cornell PGS team used the Illumina (BlueGnome, Cambridge, UK) 24SureV3 chip (aCGH) to analyse the specimens. Since 2017, the PGS team has been performing 24-chromosome aneuploidy screening with next-generation sequencing (NGS). The blastocysts were cryopreserved using the Kitazato-based vitrification method (Kuwayama 2007).

Study variables

IVF cycles were divided into the five Society for Assisted Reproductive Technology (SART) age groups: <35, 35–37, 38–40, 41–42 and >42 y.o. Euploidy rates and LBRs were compared between different durations of stimulation (<10, 10–12 and ≥13 days), total gonadotropin dosages (<4000, 4000–6000 and >6000 IU), numbers of oocytes retrieved (<10, 10–19 and ≥20 oocytes), peak E2 levels (<2000, 2000–3000 and >3000 pg/mL) and sizes of the largest follicle on the day of trigger (<20 and ≥20 mm). Of note, the follicle size was the mean of the largest two perpendicular diameters assessed by transvaginal ultrasound. The euploidy rate per cycle was calculated by dividing the number of euploid embryos, which have 46 chromosomes, by the total number of biopsied embryos.

Statistical analysis

Categorical variables were compared with the Chi-square (χ^2) and Fisher's exact tests. The odds ratios (ORs) with 95% confidence intervals (CIs) were calculated and controlled for confounding factors. We accounted for repeated measures using generalised estimating equations. Continuous variables were tested for normality. They were expressed as mean ± standard deviation, and parametric data were compared using the analysis of variance (ANOVA) test. $P < 0.05$ was considered statistically significant. Data analysis was performed with STATA statistical software version 14 (StataCorp LP).

Table II Clinical outcomes of IVF/PGT-A cycles.

	Euploidy rates (%) in women of different age groups (years)				
Age (# embryos)	**<35 (*n* = 3469)**	**35–37 (*n* = 2694)**	**38–40 (*n* = 3155)**	**41–42 (*n* = 1823)**	**>42 (*n* = 1157)**
Duration of stimulation (days)					
<10 (*n* = 882 cycles)	54.4	44.1	33.7	18.2	10.8
10–12 (*n* = 1092 cycles)	55.2	45.5	30.9	18.6	6.9
>12 (*n* = 256 cycles)	60.9	44.2	35.5	16.6	8.5
P value	NS	NS	NS	NS	NS
Gonadotropin dosages (IU)					
<4000 (*n* = 1414 cycles)	55.6	44.5	34.0	18.4	8.7
4000–6000 (*n* = 646 cycles)	52.9	47.3	30.2	16.7	9.7
>6000 (*n* = 170 cycles)	62.3	38.4	32.4	22.6	5.1
P value	NS	NS	NS	NS	NS
# oocytes retrieved					
<10 (*n* = 712 cycles)	59.4	44.1	29.9	16.9	7.3
10–19 (*n* = 1102 cycles)	55.2	44.8	32.9	19.8	10.3
≥20 (*n* = 416 cycles)	53.4	45.6	36.4	17.2	7.4
P value	NS	NS	NS	NS	NS
Peak E2 levels (pg/mL)					
<2000 (n = 1133 cycles)	55.7	46.2	31.2	16.5	8.8
2000–3000 (*n* = 676 cycles)	55.4	44.7	33.0	21.2	8.7
>3000 (*n* = 421 cycles)	54.8	41.8	35.1	18.2	7.5
P value	NS	NS	NS	NS	NS
Largest follicle size (mm)					
<20 (*n* = 1165 cycles)	55.6	44.6	34.2	16.6	8.2
≥20 (*n* = 1065 cycles)	55.1	45.0	30.3	19.8	8.9
P value	NS	NS	NS	NS	NS

The euploidy rates at different durations of stimulation, gonadotropin dosages, numbers of oocytes retrieved, peak estradiol levels and sizes of the largest follicles on the day of trigger in women who underwent IVF/PGT-A cycles. NS: not significant.

Results

A total of 2230 IVF/PGT-A cycles in which 12 298 embryos were analysed for ploidy status followed by 930 single FET cycles were included. The demographic parameters and ploidy data are summarised in Table I. There was no significant difference in parity or body mass index between the five age groups. The number of embryos biopsied, euploidy rates and number of euploid embryos were highest in the youngest age group and declined gradually with women's age (Table I and Fig. 1A). The prevalence of cycles in which no euploid embryo was obtained was subsequently the lowest in women younger than 35 y.o. (5.2%) and the highest in women older than 42 y.o. (74%) ($P < 0.001$) (Fig. 1B).

Different gonadotropin dosages were associated with comparable euploidy rates in all age groups (Table II). In the youngest group (age < 35 y.o., $n = 3469$ embryos), the prevalence of euploid embryos was 55.6% for the lowest gonadotropin dosage (<4000 IU), 52.9% for 4000–6000 IU and 62.3% for >6000 IU ($P = 0.3$). Similarly, the euploidy rates at different gonadotropin dosages ranged from 38.4 to 44.5% in women aged 35–37 y.o. ($n = 2694$ embryos) ($P = 0.3$), 30.2 to 34% in patients aged 38–40 y.o. ($n = 3155$ embryos) ($P = 0.2$), 16.7 to 22.6% in those aged 41–42 y.o. ($n = 1823$ embryos) ($P = 0.3$), and 5.1 to 8.7% in women older than 42 y.o. ($n = 1157$ embryos) ($P = 0.3$) (Table II).

The duration of stimulation did not affect the euploidy rates in any age groups (Table II). In the youngest patients (<35 y.o.), the euploidy rate was 54.4% for women who had stimulation for 10 days, 55.2% for 10–12 days and 60.9% for >12 days ($P = 0.2$). Similarly, the oldest patients (>42 y.o.) had comparable euploidy rates, ranging from 6.9 to 10.8% at various durations of stimulation ($P = 0.2$) (Table II).

The response to stimulation as assessed by the number of oocytes retrieved and peak E2 levels was not associated with the chromosomal status of embryos in any age groups (Table II). For instance, the retrieval of ≥20 oocytes was associated with 53.4% euploidy rates compared to 59.4% for <10 oocytes in women younger than 35 y.o. ($P = 0.2$). Euploidy rates ranged between 7.3 and 10.3% in women older than 42 y.o. at different numbers of oocytes retrieved ($P = 0.4$). Elevated peak E2 levels (>3000 pg/mL) were associated with comparable euploidy rates with E2 < 2000 pg/mL in all age groups: 54.8 vs. 55.7% ($P = 0.9$) in women <35 y.o., 35.1 vs. 31.2% ($P = 0.4$) in women aged 38–40 y.o. and 7.5 vs. 8.8% ($P = 0.8$) in women older than 42 y.o.

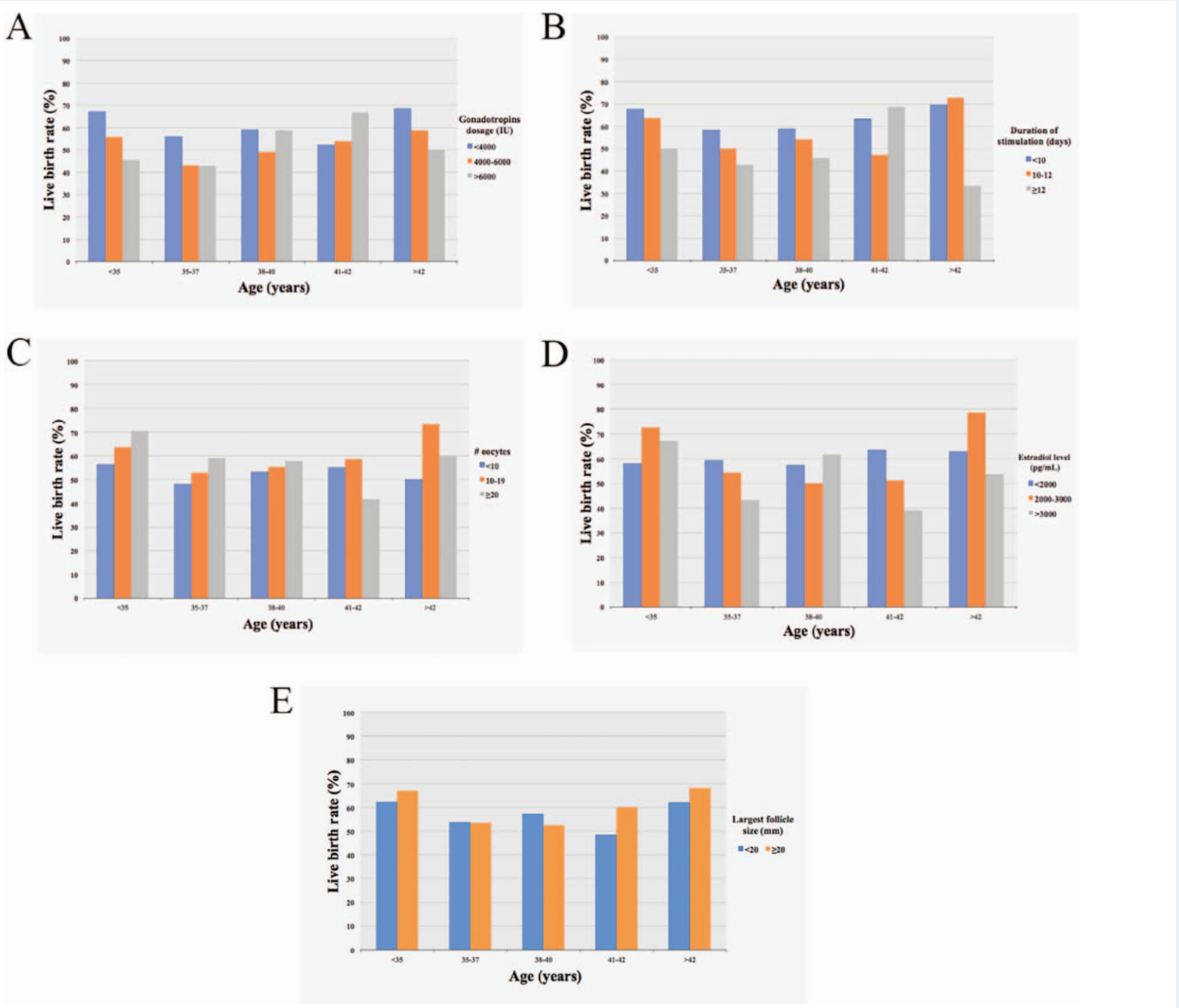

Figure 2 Live birth rates according to ovarian stimulation methods and outcomes in different age groups of women. There was no significant difference in LBRs after single frozen-thawed euploid embryo transfer cycles in which patients had different (**A**) gonadotropin dosages, (**B**) durations of stimulation, (**C**) numbers of oocytes retrieved, (**D**) peak estradiol levels or (**E**) sizes of the largest follicle on the day of the ovulation trigger during their ovarian stimulation cycles.

When cycles were stratified according to the largest follicle size on the day of trigger, cycles in which follicles were ≥20 mm were associated with comparable euploidy rates with cycles in which follicles were <20 mm: 55.1 vs. 55.6% ($P = 0.8$) in women younger than 35 y.o., 30.3 vs. 34.2% ($P = 0.08$) in women aged 38–40 y.o. and 8.9 vs. 8.2% ($P = 0.7$) in women older than 42 y.o. (Table II).

The effects of ovarian stimulation and oocyte yield on the implantation and survival potential of euploid embryos were also evaluated (Fig. 2). In the youngest group (age < 35 y.o.), LBRs ranged from 45.4 to 67.3% ($P = 0.1$) in cycles of different gonadotropin dosages (Fig. 2A). Between these groups, the LBRs ranged from 49 to 59.1% ($P = 0.2$) in women aged 38–40 y.o. and from 50% to 69.7% ($P = 0.7$) in women older than 42 y.o. (Fig. 2A). In addition, there was no significant difference in the LBRs between groups with different numbers of oocytes retrieved, peak E2 levels, durations of stimulation or sizes of the largest follicle on the day of trigger (Fig. 2).

Discussion

This study evaluated the effects of ovarian stimulation on embryo ploidy rates and LBRs after the transfer of euploid embryos. Our findings indicate that the number of oocytes retrieved, peak E2 level, duration of stimulation, total gonadotropin dosage and size of the largest follicle on the day of trigger all do not influence either embryo ploidy rates or LBRs of euploid embryos within categories of the woman's age at retrieval. The current study also shows the steep decrease with age in euploidy rates in women older than 35 years (Fig. 1A). The number of biopsied embryos also decreased with age (Table I). These two factors resulted in the positive correlation between a woman's age and the percentage of cycles in which no euploid embryo was obtained, especially for women older than 37 y.o. (Fig. 1B).

Previous studies investigating the effects of exogenous gonadotropins and high oocyte yield on oocyte quality, embryo ploidy and

implantation potential present conflicting data (Golbus 1981; Vogel and Spielmann 1992; Jackson *et al*. 1998; Katz-Jaffe *et al*. 2005; Baart *et al*. 2007; Gianaroli *et al*. 2010; Labarta *et al*. 2012; Tur-Kaspa 2012; Hong *et al*. 2019). Jackson *et al*. reviewed 483 IVF cycles and suggested that higher E2 levels and a higher number of oocytes retrieved are associated with increased embryo multinucleation and lower clinical pregnancy rates (Jackson *et al*. 1998). A small study by Baart *et al*. including women younger than 38 y.o. used fluorescence *in situ* hybridisation (FISH) to compare the ploidy rates of cleavage-stage embryos generated after a mild stimulation regimen with those obtained after a conventional high-dose exogenous gonadotropin regimen (Baart *et al*. 2007). The latter was associated with a greater number of oocytes and embryos but also a higher aneuploidy rate compared to the former, leading to a comparable number of euploid embryos (Baart *et al*. 2007). Katz-Jaffe *et al*. also suggested that a higher daily gonadotropin dose during IVF is associated with a greater number of meiotic errors compared to a lower daily gonadotropin dose (Katz-Jaffe *et al*. 2005). Vogel *et al*. analysed the chromosomes of pronuclei from mouse zygotes and reported an increased rate of aberrations in the oocyte-derived nuclei following superovulation compared to spontaneous ovulation (Vogel and Spielmann 1992). Conversely, Golbus *et al*. reported comparable mouse oocyte aneuploidy rates between oocytes obtained after superovulation and those from spontaneous ovulation (Golbus 1981). Hong *et al*. also reported comparable aneuploidy rates between 369 natural cycles and 2846 gonadotropin-stimulated IVF cycles (Hong *et al*. 2019). In addition, Labarta *et al*. conducted a prospective study to compare the aneuploidy rates in oocyte donors (mean age = 25.4 ± 4.0 years) between unstimulated and stimulated IVF cycles (Labarta *et al*. 2012). Of the 51 women who underwent unstimulated cycles, 46 also completed stimulated cycles. Embryo biopsy was performed at the cleavage stage, and FISH was used for chromosomal analysis. There was no significant difference in the aneuploidy rates between the unstimulated cycles (34.8%) and stimulated cycles (40.6%) ($P = 0.45$) (Labarta *et al*. 2012). The authors could not extrapolate these interesting results to older or infertile women because they only included young oocyte donors. Our findings are consistent with their reassuring data and, most importantly, include infertile women in all age categories who were high or poor responders and received different gonadotropin dosages.

The impact of ovarian stimulation and oocyte yield on the cumulative LBR can be interpreted as an indirect indicator of their impact on embryo development and implantation potential. For instance, a large multicenter retrospective study followed 14 469 patients for at least 2 years after their retrieval cycles to assess the outcomes of fresh and frozen cycles (Polyzos *et al*. 2018). They reported a steady increase in the cumulative LBR, which did not reach a plateau, with the increase in the number of oocytes retrieved (Polyzos *et al*. 2018). The positive correlation between oocyte yield and cumulative LBR has also been confirmed by other studies (Ji *et al*. 2013; Drakopoulos *et al*. 2016). A high number of retrieved mature oocytes (>20 mature oocytes) was also correlated with comparable implantation and ongoing pregnancy rates of euploid embryos to those with a lower number of mature oocytes (6–10 and 10–20 mature oocytes) (Unal *et al*. 2009). These results suggest that high oocyte yield is not embryotoxic, which is consistent with our data that showed comparable aneuploidy rates and LBRs between groups with different oocyte yields.

Given that stimulation does not seem to affect ploidy rates, the reported differences in euploidy rates among young oocyte donors from 42 centres could be related to other factors such as culture conditions (e.g. variations in PH or temperature) (Pickering *et al*. 1990; Almeida and Bolton 1995; Munne *et al*. 1997; Munne *et al*. 2017). It is important to note that the majority of patients included in our study in the high E2 group (>3000 pg/mL) had E2 levels that ranged between 3000 and 5000 pg/mL, with a mean level of 3703 ± 658 pg/mL. Furthermore, most women in the high oocyte yield group (≥20 oocytes) had 20–35 retrieved oocytes (mean 25 ± 6 oocytes). Therefore, the conclusions of this single-centre study must be interpreted with caution, as we rarely encounter women whose E2 is >5000 pg/mL or who have >35 oocytes (Valbuena *et al*. 2001). Similarly, we cannot attest to the safety of administering hCG at follicles >22 mm because the overwhelming majority of our patients included in this study were triggered when the average size of the largest follicle was ≤22 mm.

The reassuring findings of this study should not be misinterpreted as a promotion to aggressively stimulate patients, especially since extreme ovarian responses are linked to higher risks of complications such as intra-abdominal bleeding, ovarian torsion and OHSS (Bodri *et al*. 2008; Steward *et al*. 2014). It must also be emphasised that using GnRH agonist triggers followed by a freeze-all policy minimises, but does not eliminate, the risk of OHSS (Fatemi *et al*. 2014; Ling *et al*. 2014). Another reason to avoid aggressive stimulation is the finding that some women undergoing GnRH antagonist IVF stimulation protocols may fail to respond to the GnRH agonist trigger, and thus may require hCG to trigger final oocyte maturation (Meyer *et al*. 2015).

In conclusion, this study reassures clinicians and patients that the total gonadotropin dosage, duration of ovarian stimulation, size of the largest follicle on the day of trigger, peak estradiol level and oocyte yield, within certain ranges, do not appear to influence aneuploidy rates or the viability of the euploid embryos, in any age category.

Acknowledgements

The study was presented in oral format at the 34th Annual Meeting of the European Society of Human Reproduction and Embryology, Barcelona, Spain, 1–4 July 2018.

Authors' roles

M.I., C.C., A.R., B.M., V.G., X.Q., C.Z., K.X., Z.R.: conception and design of study, acquisition of data, analysis of data and approval of the final version. M.I.: drafting the manuscript. C.C., A.R., B.M., V.G., X.Q., C.Z., K.X., Z.R.: revising the manuscript critically for intellectual content.

Funding

The authors received no specific funding for this work.

Conflict of interest

The authors have nothing to disclose.

References

Almeida PA, Bolton VN. The effect of temperature fluctuations on the cytoskeletal organisation and chromosomal constitution of the human oocyte. *Zygote* 1995;**3**:357–365.

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007;**22**: 980–988.

Bodri D, Guillen JJ, Polo A, Trullenque M, Esteve C, Coll O. Complications related to ovarian stimulation and oocyte retrieval in 4052 oocyte donor cycles. *Reprod Biomed Online* 2008;**17**:237–243.

Drakopoulos P, Blockeel C, Stoop D, Camus M, de Vos M, Tournaye H, Polyzos NP. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? *Hum Reprod* 2016;**31**:370–376.

Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril* 2008;**89**:84–91.

Fatemi HM, Popovic-Todorovic B, Humaidan P, Kol S, Banker M, Devroey P, Garcia-Velasco JA. Severe ovarian hyperstimulation syndrome after gonadotropin-releasing hormone (GnRH) agonist trigger and "freeze-all" approach in GnRH antagonist protocol. *Fertil Steril* 2014;**101**:1008–1011.

Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT Jr. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* 2014;**101**:656–663 e651.

Gianaroli L, Magli MC, Cavallini G, Crippa A, Capoti A, Resta S, Robles F, Ferraretti AP. Predicting aneuploidy in human oocytes: key factors which affect the meiotic process. *Hum Reprod* 2010;**25**:2374–2386.

Golbus MS. The influence of strain, maternal age, and method of maturation on mouse oocyte aneuploidy. *Cytogenet Cell Genet* 1981;**31**:84–90.

Hong KH, Franasiak JM, Werner MM, Patounakis G, Juneau CR, Forman EJ, Scott RT Jr. Embryonic aneuploidy rates are equivalent in natural cycles and gonadotropin-stimulated cycles. *Fertil Steril* 2019;**112**:670–676.

Huang JY, Rosenwaks Z. Assisted reproductive techniques. *Methods Mol Biol* 2014;**1154**:171–231.

Jackson KV, Ginsburg ES, Hornstein MD, Rein MS, Clarke RN. Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles. *Fertil Steril* 1998;**70**:60–66.

Ji J, Liu Y, Tong XH, Luo L, Ma J, Chen Z. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. *Hum Reprod* 2013;**28**:2728–2734.

Katz-Jaffe MG, Trounson AO, Cram DS. Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. *Fertil Steril* 2005;**84**:634–643.

Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology* 2007;**67**:73–80.

Labarta E, Bosch E, Alama P, Rubio C, Rodrigo L, Pellicer A. Moderate ovarian stimulation does not increase the incidence of human embryo chromosomal abnormalities in in vitro fertilization cycles. *J Clin Endocrinol Metab* 2012;**97**:E1987–E1994.

Lee ST, Kim TM, Cho MY, Moon SY, Han JY, Lim JM. Development of a hamster superovulation program and adverse effects of gonadotropins on microfilament formation during oocyte development. *Fertil Steril* 2005;**83**Suppl 1:1264–1274.

Leridon H. Can assisted reproduction technology compensate for the natural decline in fertility with age? A model assessment. *Hum Reprod* 2004;**19**:1548–1553.

Ling LP, Phoon JW, Lau MS, Chan JK, Viardot-Foucault V, Tan TY, Nadarajah S, Tan HH. GnRH agonist trigger and ovarian hyperstimulation syndrome: relook at 'freeze-all strategy'. *Reprod Biomed Online* 2014;**29**:392–394.

Meyer L, Murphy LA, Gumer A, Reichman DE, Rosenwaks Z, Cholst IN. Risk factors for a suboptimal response to gonadotropin-releasing hormone agonist trigger during in vitro fertilization cycles. *Fertil Steril* 2015;**104**:637–642.

Munne S, Alikani M, Ribustello L, Colls P, Martinez-Ortiz PA, McCulloh DH, Referring PG. Euploidy rates in donor egg cycles significantly differ between fertility centers. *Hum Reprod* 2017;**32**:743–749.

Munne S, Magli C, Adler A, Wright G, de Boer K, Mortimer D, Tucker M, Cohen J, Gianaroli L. Treatment-related chromosome abnormalities in human embryos. *Hum Reprod* 1997;**12**:780–784.

Pickering SJ, Braude PR, Johnson MH, Cant A, Currie J. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. *Fertil Steril* 1990;**54**:102–108.

Polyzos NP, Drakopoulos P, Parra J, Pellicer A, Santos-Ribeiro S, Tournaye H, Bosch E, Garcia-Velasco J. Cumulative live birth rates according to the number of oocytes retrieved after the first ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including approximately 15,000 women. *Fertil Steril* 2018;**110**:661–670 e661.

Roberts R, Iatropoulou A, Ciantar D, Stark J, Becker DL, Franks S, Hardy K. Follicle-stimulating hormone affects metaphase I chromosome alignment and increases aneuploidy in mouse oocytes matured in vitro. *Biol Reprod* 2005;**72**:107–118.

Sachdeva K, Upadhyay D, Discutido R, Varghese MM, Albuz F, Almekosh R, Bouhafs L, Solkar S, Stevikova M, Peramo B. Low gonadotropin dosage reduces aneuploidy in human preimplantation embryos: first clinical study in a UAE population. *Genet Test Mol Biomarkers* 2018;**22**:630–634.

Steiner AZ, Jukic AM. Impact of female age and nulligravidity on fecundity in an older reproductive age cohort. *Fertil Steril* 2016;**105**: 1584–1588 e1581.

Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, Muasher SJ. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles. *Fertil Steril* 2014;**101**:967–973.

Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Hum Reprod* 2011;**26**:1768–1774.

Tur-Kaspa I. Clinical management of in vitro fertilization with preimplantation genetic diagnosis. *Semin Reprod Med* 2012;**30**: 309–322.

Unal SYH, Altin G, Candan ZN, Kumtepe Y, Kahraman S. Preimplantation genetic diagnosis (PGD) results with respect to number of mii oocyte. *Fertil Steril* 2009;**92**:S202–S203.

Valbuena D, Martin J, de Pablo JL, Remohi J, Pellicer A, Simon C. Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. *Fertil Steril* 2001;**76**:962–968.

Van der Auwera I, D'Hooghe T. Superovulation of female mice delays embryonic and fetal development. *Hum Reprod* 2001;**16**:1237–1243.

Vogel R, Spielmann H. Genotoxic and embryotoxic effects of gonadotropin-hyperstimulated ovulation of murine oocytes, preimplantation embryos, and term fetuses. *Reprod Toxicol* 1992;**6**: 329–333.