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ORIGINAL ARTICLE *Embryology*

High gonadotropin dosage does not affect euploidy and pregnancy rates in IVF PGS cycles with single embryo transfer

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STUDY QUESTION: Does high gonadotropin dosage affect euploidy and pregnancy rates in PGS cycles with single embryo transfer?

SUMMARY ANSWER: High gonadotropin dosage does NOT affect euploidy and pregnancy rates in PGS cycles with single embryo transfer.

WHAT IS KNOWN ALREADY: PGS has been proven to be the most effective and reliable method for embryo selection in IVF cycles. Euploidy and blastulation rates decrease significantly with advancing maternal age. In order to recruit an adequate number of follicles, the average dosage of gonadotropins administered during controlled ovarian stimulation in IVF cycles often increases significantly with advancing maternal age.

STUDY DESIGN, SIZE, AND DURATION: A retrospective study of SNP (Single Nucleotide Polymorphism) PGS outcome data from blastocysts biopsied on day 5 or day 6 was conducted to identify differences in euploidy and clinical pregnancy rates. Seven hundred and ninety four cycles of IVF treatment with PGS between January 2013 and January 2017 followed by 651 frozen embryo transfers were included in the study (506 patients, maternal age (y.o.) – 37.2 ± 4.31).

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 4034 embryos were analyzed (5.1 ± 3.76 per case) for euploidy status. All embryos were vitrified after biopsy, and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer cycle. All cycles were analyzed by total gonadotropin dosage (<3000 IU, 3000–5000 IU and >5000 IU), by number of eggs retrieved (1–5, 5–10, 10–15 and >15 eggs) and patient's age (<35, 35–37, 38–40 and ≥41 y.o.). Clinical pregnancy rate was defined by the presence of a fetal heartbeat at 6–7 weeks of gestation.

MAIN RESULTS AND THE ROLE OF CHANCE: Euploidy rates within the same age group were not statistically different regardless of the total dosage of gonadotropins used or the number of eggs retrieved. In the youngest group of patients (<35 y.o. – 187 IVF cycles) euploidy rates ranged from 62.3% (<3000 IU were used in the IVF cycle) to 67.5% (>5000 IU were used in the IVF cycle) (OR = 0.862, 95% CI 0.687–1.082, $P = 0.2$) and from 69.5% (1–5 eggs retrieved) to 60.0% (>15 eggs retrieved) (OR = 0.658, 95% CI 0.405–1.071, $P = 0.09$). Similar data were obtained in the oldest group of patients (≥41 y.o. – 189 IVF cycles): euploidy rates ranged from 30.7 to 26.4% (OR = 0.811, 95% CI 0.452–1.454, $P = 0.481$) when analyzed by total dosage of gonadotropins used in the IVF cycle and from 40.0 to 30.7% (OR = 0.531, 95% CI 0.204–1.384, $P = 0.19$), when assessed by the total number of eggs retrieved. Ongoing pregnancy rates were similar, not only within particular age groups, but also between different age groups regardless of the total dosage of gonadotropins used: ranging from to 63.6% (<3000 IU, < 35 y.o.) to 54.8% (>5000 IU, ≥41 y.o) (OR = 0.696, 95% CI 0.310–1.565, $P = 0.38$).

LIMITATIONS, REASONS FOR CAUTION: Retrospective study and heterogeneity of patients included.

WIDER IMPLICATIONS OF THE FINDINGS: These data are reassuring for the common practice of increasing gonadotropin dosages in PGS cycles, particularly in older woman.

STUDY FUNDING/COMPETING INTEREST(S): No formal funding has been received for this study.

TRIAL REGISTRATION NUMBER: N/A.

Key words: preimplantation genetic screening / gonadotropin dosage / IVF / CCS / euploidy rates / blastocyst morphology / blastocyst biopsy / embryo development.

Introduction

Recent scientific advances revealed that over half of human preimplantation embryos created during IVF cycles have major or minor chromosomal abnormalities (Munné *et al.*, 1995; Macklon *et al.*, 2002). The proportion of aneuploid embryos increases significantly with advancing maternal age (Staessen *et al.*, 2004).

Most women of advanced age experience decrease in ovarian reserve and hormonal dysfunction due to natural biological aging mechanisms (Levi *et al.*, 2001). The combined effect of increased aneuploidy rates and diminished ovarian response in the older patient population is manifested in an increased proportion of IVF PGS cycles where no euploid embryos are detected (Magli *et al.*, 1998).

Human FSH dose-response trials are limited (Acre *et al.*, 2014). Most of the knowledge was acquired from animal models (mainly cattle) (Armstrong *et al.*, 1994; Damiani *et al.*, 1996). In cattle, FSH dose-response has an S-shaped curve with a clear 'plateau': an increase in FSH dose over maximum response was followed by a disturbance of the ovulation process at the level of pituitary and at the ovarian level as well as down-regulation of follicular FSH receptors by high doses of the homologous ligand (Kanitz *et al.*, 2002).

In human ART, the general consensus is that gonadotropin stimulation doses greater than 450 IU per day are not recommended (Serono Gonal-f pharmacokinetics and Pharmacodynamics). While some studies assert that adverse effects of high gonadotropin dosages are seen on oocyte quality (Hansmann and El-Nahass, 1979; Shapiro *et al.*, 2001) and clinical pregnancy rates (Wennerholm *et al.*, 1997; Källén *et al.*, 2005), a wide range of recent studies refute these claims (Ji *et al.*, 2013; Steward *et al.*, 2014; Drakopoulos *et al.*, 2016).

A study published by Ata revealed no association between the number of embryos generated and aneuploidy rates (Ata *et al.*, 2012). Sekhon *et al.* (2017) in recent study reported that exogenous gonadotropins did not significantly modify the aneuploidy rates in cases with normal duration of ovarian stimulation (up to 12 days).

Given the conflicting reports and lack of guidance regarding gonadotropin dosing, our goal was to analyze clinical data to determine whether different dosages of gonadotropins influence the rates of euploidy and clinical pregnancy in IVF PGS cycles. This information may be used to adjust treatment strategy in IVF cycles with and without PGS in order to provide best medical care to the patients.

Materials and Methods

A retrospective comparative study was performed between January 2013 and January 2017. Seven hundred and ninety four cycles of IVF treatment with PGS (681 patients) were included in the study. After comprehensive chromosomal testing, 184 IVF cycles (162 patients) had only aneuploid embryos. A total of 519 patients had at least one euploid embryo and 506 of them had a total of 651 single frozen embryo transfers. Preimplantation genetic diagnosis cases for single gene disorder, translocation (balanced and unbalanced) and gender selection were excluded from the study.

All patients underwent ovarian reserve evaluation with anti-Müllerian hormone testing, antral follicle counts, and menstrual cycle day 3 FSH and estradiol levels within 6 months of the index IVF cycle. There were three stimulation protocols used: antagonist (636 IVF cycles, average age 36.59 ± 4.40), microdose Lupron flare protocol (142 IVF cycles, average age 39.38 ± 3.11), and long protocol Lupron (16 IVF cycles, average age 36.75 ± 2.89). The majority of cycles included a short course of oral contraceptives and combined FSH (GONAL-f, EMD Serono Inc., USA) and HMG (Menopur, Ferring Pharmaceuticals Inc., USA). Antagonist cycles began with GnRHa with a 12–14 mm lead follicle. Leuprolide (Oakwood Laboratories, L.L.C., USA) 2 mg or human chorionic gonadotropin (Pregnyl, Baxter Oncology GmbH, Germany) 10 000 units were used for triggering ovulation. Oocyte retrieval occurred 36 h after trigger.

Patients were divided into three, approximately equal, gonadotropin dosage groups (<3000 IU, 3000–5000 IU, >5000 IU per IVF cycle), into four groups by number of eggs retrieved (1–5, 6–10, 11–15 and >15 eggs), and into four age groups corresponding to the groupings used by SART (<35, 35–37, 38–40 and ≥41 y.o.).

Embryos were cultured in MINC benchtop incubators at 37°C in a low oxygen culture system in a humidified atmosphere (7% CO_2 and 5% O_2, 88% N_2). Quinn's Advantage sequential culture medium supplemented with 10% (v/v) Serum Protein Substitute under mineral oil was used according to the manufacturer's recommendations (Sage In Vitro Fertilization, Inc., USA).

A total of 4034 embryos were analyzed for euploidy rates and blastocyst morphology: 1782 (44.2%) embryos were biopsied on Day 5, and 2252 (55.8%) embryos were biopsied on Day 6. After genetic analysis 12 embryos (0.3%) were reported as 'No Result' and were excluded from the study.

Morphology of blastocysts was evaluated independently by two embryologists using the Gardner classification (Gardner and Schoolcraft, 1999) before trophectoderm biopsy. Embryos were divided into four groups based on blastocyst quality: good (AA/AB/BA—a total of 1816 embryos), fair (BB—a total of 1381 embryos), borderline fair (B-/-B—a total of 781 embryos) and poor quality embryos (C/C—a total of 56 embryos). Poor quality blastocysts were biopsied as exceptions only per clinician or patient request (three FETs were performed).

Usable blastocyst formation rate was calculated as proportion of zygotes that became good or fair quality blastocysts and were used during an IVF cycle (biopsied on Days 5/6 and/or frozen, transferred).

Euploidy status of the embryos was assessed in commercial reference laboratory by SNP (Illumina HumanCytoSNP-12 DNA BeadChips in combination with an informatics-based algorithm, Parental Support™, Natera Inc).

Once determined a euploid embryo was available for transfer, a hormone replacement frozen embryo transfer cycles was initiated. Clinical pregnancy was defined as an intrauterine gestational sac at 6–7 weeks estimated gestational age with fetal pole and cardiac activity via sonography. Ongoing pregnancy was defined as a viable pregnancy after 8 weeks gestation. Live birth data was obtained in all IVF cycles completed in 2013–2015.

The study protocol was approved by the Ethics Committee of the Reproductive Science Center of the San Francisco Bay Area and was performed according to the Declaration of Helsinki for Medical Research.

The study was designed to have sufficient statistical power to detect at least 10% difference between study groups. The probability of a type-I error (Alpha) was set to 0.05, the probability of a type-II error (not detecting a difference when one actually exists) was set to 0.2 (beta cut-off). So, for the dichotomous endpoint (Independent Sample Study) at least 774 subjects should be included. In our study, we included 794 IVF cycles (to evaluate the effect of different gonadotropin dosages on ongoing pregnancy rate) and 4034 blastocysts (to evaluate the effect of different gonadotropin dosages on euploidy rates).

Chi-square analysis was used to assess the difference in categorical values. Multiple logistic regression analysis was used to evaluate the association between pregnancy rates, euploidy rates and maternal age (y.o.), total gonadotropin dosage (IU per cycle) number of egg retrieved (per cycle). Maternal age, total gonadotropin dosage, and number of egg retrieved were fitted as continuous values in Multiple logistic regression analysis. Odds ratio in 2 × 3 contingency tables was calculated between the two most distant categorical variables. Continuous values in Tables II and IV were presented as means with standard deviation with ANOVA analysis. The statistical analysis was performed using R statistical software version 3.3.1—The R Foundation for Statistical Computing. A *P*-value of <0.05 was considered statistically significant.

Results

A total of 12925 oocytes (16.28 ± 9.53 per cycle) were retrieved, and 9915 MII oocytes (12.7 ± 8.6 per cycle) were inseminated by Intracytoplasmic Sperm Injection (ICSI). A total of 8449 (10.7 ± 7.1 per cycle) zygotes were obtained and cultured to the blastocyst stage.

The average number of oocytes aspirated in PGS cycles decreased gradually with advancing maternal age: from 23.5 ± 7.2 in a group of patients under 35 y.o. to 14.15 ± 6.11 in a group of patients over 41 y.o. Conversely, the total gonadotropin dosage used in IVF PGS cycles increased with advancing maternal age: from 3332 ± 1241 IU in a group of patients under 35 y.o. to 5027 ± 1201 IU in a group of patients over 41 y.o.

Euploidy rates in the different age groups are shown in Fig. 1. Euploidy rates in the group of young patients (<35 y.o. – 187 IVF cycle) were similar regardless of gonadotropin dosing. The percent of euploid blastocysts were 62.32% (506/812) in the group of patients with low gonadotropin dose (<3000 IU), 58.78% (298/507) in the group of patients with medium dose (3000–5000 IU), and 67.50% (27/40) in the group of patients with high gonadotropin dose (>5000 IU). The difference in euploidy rates between the three gonadotropin dosage groups was not statistically significant (OR = 0.638, 95% CI 0.322–1.266, *P* = 0.196).

Similar data were obtained in the group of patients aged 35–37 y.o. (179 IVF cycles): euploidy rates ranged from 54.12% (46/85) to 57.38% (245/427) (OR = 0.876, 95% CI 0.549–1.399, *P* = 0.58). In the group of patients 38–40 y.o. (239 IVF cycles)—euploidy rate ranged from 36.55% (72/197) to 47.92% (173/361) (OR = 0.626, 95% CI 0.438–0.894, *P* = 0.01). In the group of patients of advanced maternal age (≥41 y.o. – 189 IVF cycles) euploidy rates were not statistically different and ranged from 26.40% (52/197) to 30.67% (23/75) (OR = 0.811, 95% CI 0.452–1.454, *P* = 0.481).

Analysis of euploidy rates by the number of oocytes retrieved in IVF PGS cycles revealed no association between the total number of retrieved oocytes and chromosomal status of the embryo in all age groups (Fig. 2). In young patients (<35 y.o.), euploidy rates were not statistically different regardless of the number of eggs retrieved during controlled ovarian stimulation: 100% (2/2) in the group of patients with 1–5 eggs retrieved, 69.51% (57/82) in the group of patients with 6–10 eggs retrieved, 63.06% (140/222) in the group with 11–15 eggs retrieved and 60.02% (632/1053) in the group of patients with over 15 eggs retrieved (OR = 0.658, 95% CI 0.405–1.071, *P* = 0.09).

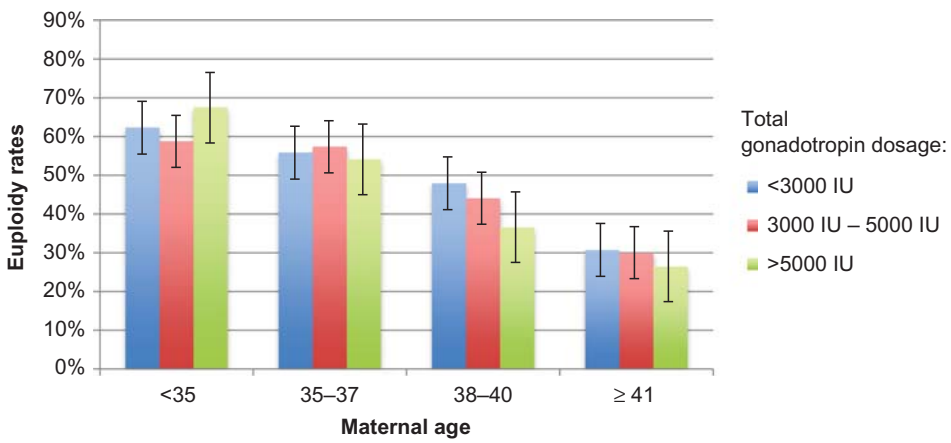

Figure 1 Comparison of euploidy rates after administration of different dosages of gonadotropins in IVF PGS cycles.

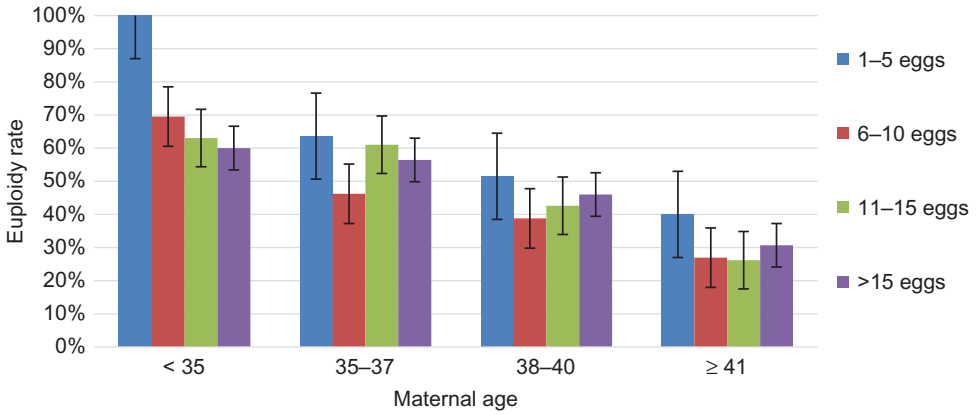

Figure 2 Comparison of euploidy rates by the number of oocytes retrieved during controlled ovarian stimulation in IVF PGS cycles.

Table I Multiple logistic regression analysis of the association between chromosomal status and total gonadotropins dosages in IVF PGS cycles.

		Coeff.	Std. error	*P*-value	Odds ratio	CI Lower limit	CI Upper limit
Under 35 y.o.	(Intercept)	0.47143	0.27	0.08	1.60	0.95	2.71
	Number of eggs	0.00308	0.01	0.59	1.00	0.99	1.01
	Total gonadotropin dosage	−0.00003	0.00	0.62	1.00	1.00	1.00
35–37 y.o.	(Intercept)	0.30303	0.30	0.32	1.35	0.75	2.45
	Number of eggs	0.00016	0.01	0.98	1.00	0.99	1.01
	Total gonadotropin dosage	−0.00001	0.00	0.79	1.00	1.00	1.00
38–40 y.o.	(Intercept)	0.17246	0.31	0.58	1.19	0.65	2.18
	Number of eggs	−0.00066	0.01	0.93	1.00	0.99	1.01
	Total gonadotropin dosage	−0.00010	0.00	0.048*	1.00	1.00	1.00
over 41 y.o.	(Intercept)	−0.58156	0.45	0.19	0.56	0.23	1.35
	Number of eggs	0.00738	0.01	0.47	1.01	0.99	1.03
	Total gonadotropin dosage	−0.00010	0.00	0.18	1.00	1.00	1.00

Coeff. = coefficient expressed in logits; CI = 95% confidence interval for the odds ratio.
Number of Fisher Scoring iterations: 4. *$P < 0.05$.

Similar results were shown in the group of patients 35–37 y.o.: euploidy rates ranged from 63.64% (7/11) in the group of patients with 1–5 eggs retrieved to 56.43% (408/723) in the group of patients with over 15 eggs retrieved (OR = 0.740, 95% CI 0.215–2.551, $P = 0.632$). In patients over 41 y.o. euploidy rates ranged from 26.16% (45/172) in the group of patients with 11–15 eggs retrieved to 40.00% (8/20) in the group of patients with >15 eggs retrieved (OR = 0.531, 95% CI 0.204–1.384, $P = 0.19$).

Analysis of the data had shown moderate positive correlation between patient age and total gonadotropin dosage used in IVF PGS cycles ($r = 0.397$), so multiple regression analysis was performed separately in each age group. Statistically significant impact of high gonadotropin dosages on euploidy status of the embryos was detected only in a group of patients 38–40 y.o. ($P = 0.04839$) (Table I).

In all age groups, the usable blastocyst rate in PGS cycles where <3000 IU of gonadotropins were used was higher than in PGS cycles where over 5000 IU of gonadotropins were used. This difference reached statistical significance only in the group of patients 35–37 y.o. (OR = 1.417, 95% CI 1.047–1.918, $P = 0.024$). The difference in blastulation rates had a tendency to decrease with advancing maternal age. In the group of patients ≥41 y.o. the difference in blastulation rates varied in a narrow range from 35.61% to 38.27% (OR = 1.121, 95% CI 0.8–1.571, $P = 0.507$) (Table II).

We observed that regardless of total gonadotropin dosage administered in IVF PGS cycle, the proportion of good, fair, borderline fair and poor quality embryos available for biopsy remained similar. At the same time, the proportion of good quality embryos was moderately affected by high gonadotropin dosage: the proportion of good quality

Table II Characteristics of IVF PGS cycles by maternal age and total gonadotropin dosage.

Maternal age/ gonadotropin dosage (IU)	Number of IVF cycles	FSH (mIU/ml)	AMH (ng/ml)	Gonadotropin dosage per IVF cycle	Duration of stimulation (days)	Average number of aspirated oocytes per IVF cycle	Average number of biopsied embryos per IVF cycle	Average number of euploid embryos per IVF cycle	Usable blast. rate, %
<35 y.o.	187	5.45 ± 3.00	5.02 ± 4.20	3191 ± 1111	11.29 ± 1.59	19.94 ± 9.54	7.34 ± 4.14	4.53 ± 3.01	58.99
<3000	100	5.24 ± 2.83	6.59 ± 4.71	2382 ± 454	10.81 ± 1.28	23.28 ± 9.77	8.17 ± 4.43	5.13 ± 3.25	59.90
3000–5000	76	5.65 ± 3.29	3.20 ± 2.28	3860 ± 577	11.61 ± 1.59	16.72 ± 7.94	6.76 ± 3.59	4.04 ± 2.62	58.14
>5000	11	5.86 ± 2.72	1.86 ± 0.85	5925 ± 519	13.45 ± 1.81	11.73 ± 5.41	3.73 ± 2.05	2.45 ± 1.63	52.56
35–37 y.o.	179	6.17 ± 3.19	3.50 ± 2.88	3864 ± 1384	11.50 ± 1.70	16.97 ± 9.66	5.64 ± 4.10	3.19 ± 2.69	49.27
<3000	63	5.57 ± 2.99	5.05 ± 2.80	2461 ± 404	10.63 ± 1.63	21.71 ± 10.46	7.81 ± 4.47	4.35 ± 3.10	50.46
3000–5000	85	6.50 ± 2.91	3.03 ± 2.77	4102 ± 582	11.61 ± 1.36	15.49 ± 8.01	5.06 ± 3.65	2.91 ± 2.37	49.71
>5000	31	6.47 ± 4.14	1.33 ± 0.82	6061 ± 913	12.97 ± 1.63	11.35 ± 5.53	2.81 ± 1.35	1.61 ± 1.31	41.83
38–40 y.o.	239	7.03 ± 2.58	2.66 ± 2.66	4375 ± 1493	11.50 ± 1.84	14.63 ± 9.18	4.37 ± 3.06	1.93 ± 1.78	43.87
<3000	48	5.72 ± 2.35	5.19 ± 4.00	2438 ± 405	10.79 ± 1.24	24.10 ± 11.77	7.48 ± 3.41	3.58 ± 2.57	46.44
3000–5000	120	7.45 ± 2.63	2.34 ± 1.75	4082 ± 598	11.03 ± 1.66	13.88 ± 7.21	3.98 ± 2.67	1.80 ± 1.74	41.55
>5000	71	7.22 ± 2.38	1.30 ± 0.80	6181 ± 913	12.79 ± 1.84	9.48 ± 4.02	2.93 ± 1.69	1.04 ± 0.99	45.32
≥41 y.o.	189	7.26 ± 2.83	2.39 ± 2.26	4828 ± 1307	11.56 ± 1.71	14.10 ± 8.03	3.36 ± 2.43	1.01 ± 1.34	37.46
<3000	14	6.21 ± 2.36	6.52 ± 5.63	2504 ± 496	10.29 ± 1.20	22.29 ± 10.07	5.36 ± 1.98	1.79 ± 1.12	38.27
3000–5000	98	7.26 ± 2.89	2.60 ± 1.85	4220 ± 584	10.94 ± 1.26	14.61 ± 7.38	3.74 ± 2.7	1.16 ± 1.48	38.35
>5000	77	7.49 ± 2.85	1.44 ± 0.96	6024 ± 911	12.57 ± 1.76	11.95 ± 7.38	2.51 ± 1.71	0.68 ± 0.59	35.61
Grand Total	794	6.57 ± 2.95	3.36 ± 3.25	4089 ± 1466	11.47 ± 1.72	16.28 ± 9.45	5.11 ± 3.76	2.61 ± 2.36	48.05

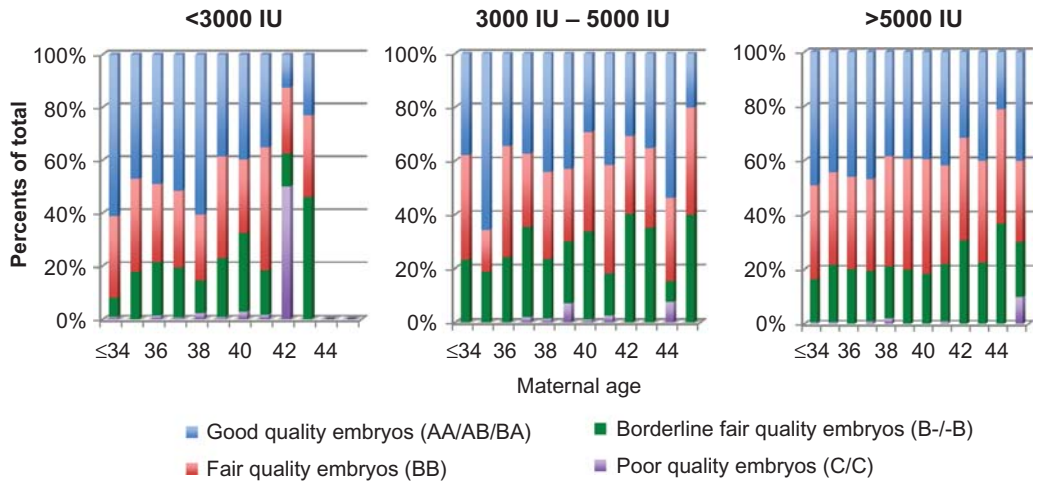

Figure 3 Distribution of embryos available for biopsy by morphology after administration of different dosages of gonadotropins in IVF PGS cycles.

embryos decreased from 48.9% (500/1022) in a group <3000 IU to 38.3% (199/519) in the group >5000 IU were administered (OR = 0.649, 95% CI 0.523–0.805, $P = 0.01$) (Fig. 3).

Ongoing pregnancy rates after administration of different dosages of gonadotropins were not statistically different within all study groups and ranged from 45.5% (5/11) to 63.6% (68/107) in the group of patients under 35 y.o. (a total of 196 SETs, OR = 0.478, 95% CI 0.137–1.669, $P = 0.239$), from 60% (40/67) to 71% (17/24) in the group of patients 35–37 y.o. (a total of 165 SETs, OR = 0.610, 95% CI 0.223–1.669, $P = 0.333$), from 59% (57/97) to 69% (34/49) in the group of patients 38–40 y.o. (a total of 190 SETs, OR = 0.629, 95% CI 0.303–1.304, $P = 0.211$), and from 43% (6/14) to 62% (34/55) in a group of patients over 41 y.o (a total of 100 SETs, OR = 0.463, 95% CI 0.141–1.523, $P = 0.199$) (Fig. 4).

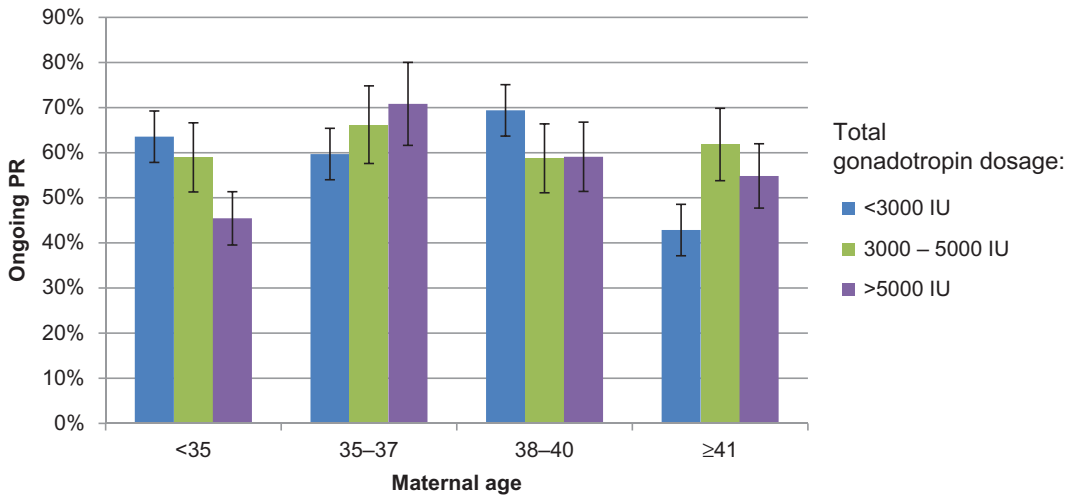

Figure 4 Comparison of ongoing clinical pregnancy rates after administration of different dosages of gonadotropins in IVF PGS cycles.

Table III Multiple logistic regression analysis of the association between ongoing pregnancy and patient's age, number of eggs recovered, total gonadotropins dosage in IVF PGS cycles.

	Coeff.	Std. Error	*P*-value	Odds ratio	CI lower limit	CI upper limit
(Intercept)	1.28524	0.94	0.17	3.62	0.59	23.21
Patient's age	0.00216	0.02	0.93	1.00	0.95	1.05
Number of eggs	−0.00049	0.01	0.97	1.00	0.98	1.02
Total gonadotropin dosage	−0.00001	0.00	0.87	1.00	1.00	1.00

Coeff., coefficient expressed in logits; CI = 95% confidence interval for the odds ratio;
Null deviance: 611.99 on 589 degrees of freedom. Residual deviance: 611.96 on 586 degrees of freedom
AIC: 619.96 Number of Fisher Scoring iterations: 4.

Similar results were obtained with multiple logistic regression analysis: patient's age, number of oocytes retrieved and total gonadotropin dosage does not affect ongoing pregnancy rates in IVF PGS cycles with single embryo transfer (coefficients of multiple logistic regression: 0.00216, −0.000049 and −0.00001, respectively) (Table III).

The increase in the total dose of gonadotropins used in IVF PGS cycles had minimal effect on clinical outcomes in the group of younger patients (<38 y.o.), as well as in the group of patients ≥38 y.o. (Table IV).

Discussion

Advances in comprehensive chromosomal screening technology have significantly improved clinical outcomes in IVF PGS cycles in recent years. Improved clinical outcomes stimulated the wider acceptance of PGS in ART treatment, especially for the older patient population (≥38 y.o.). However, due to a significant decrease in euploidy rates, many more oocytes and subsequently good quality blastocysts are needed in order to obtain at least one euploid embryo with advancing maternal age.

Reports on the effects of conventional versus mild stimulation on human embryo quality and clinical outcomes in many instances present conflicting data (Wang *et al.*, 2008; Andersen *et al.*, 2017). In a recent report, Munne and collaborators presented evidence that some embryonic chromosomal abnormalities may have a partly iatrogenic origin (Munné *et al.*, 2017). Embryo culture protocols, culture conditions and/or stimulation protocols along with medications dosage may alter the level of post-meiotic chromosomal abnormalities in the pre-implantation embryos.

The effect of high gonadotropin doses is still poorly understood, especially on a cellular level. Previous studies suggested that high FSH doses might be associated with meiotic divisions errors (Munne *et al.*, 1997; Baart *et al.*, 2007). While this is a reasonable assumption, considering the high number of the recruited antral follicles and the additional stress on intracellular cytoplasmic machinery, our data support those findings reported by Labarta that moderate stimulation does not affect euploidy rates in IVF PGS cycles (Labarta *et al.*, 2012). Our results support findings reported by Ata showing that euploidy rates

Table IV Characteristics of IVF PGS cycles in a group of patients under 38 y and over 38 y after administration of different dosages of gonadotropins in IVF PGS cycles.

	Under 38 y.o.				Over 38 y.o.			
	<3000 IU	3000–5000 IU	>5000 IU	*P*-value	<3000 IU	3000–5000 IU	>5000 IU	*P*-value
Number of SETs	174	152	35	NA	63	152	75	NA
AMH (ng/ml)	5.81 ± 4.32	3.28 ± 2.73	1.38 ± 0.82	<0.05	6.23 ± 4.88	2.62 ± 1.92	1.51 ± 0.80	<0.05
FSH (mIU/ml)	5.39 ± 2.82	6.25 ± 2.90	6.40 ± 3.92	NS	6.07 ± 2.06	7.35 ± 2.41	7.55 ± 2.72	NS
Peak E2 (pg/ml)	3710 ± 1553	3343 ± 1434	2456 ± 979	NS	3913 ± 1408	3341 ± 1105	2520 ± 1002	NS
Duration of stimulation (days)	10.74 ± 1.37	11.65 ± 1.47	12.86 ± 1.52	NS	10.70 ± 1.19	11.15 ± 2.62	12.63 ± 1.81	NS
Gonadotropin dosage per IVF cycle	2444 ± 435	3894 ± 600	6142 ± 858	<0.05	2456 ± 439	4090 ± 592	6112 ± 904	<0.05
Number of aspirated oocytes	3987	2661	398	NA	1588	2281	853	NA
Average number of aspirated oocytes per IVF cycle	22.91 ± 10.26	17.51 ± 8.72	11.37 ± 5.58	<0.05	25.21 ± 12.80	15.01 ± 8.21	11.37 ± 5.61	<0.05
Number of biopsied embryos	1436	1011	126	NA	474	721	247	NA
Average number of embryos for biopsy per IVF cycle	8.25 ± 4.27	6.65 ± 3.73	3.60 ± 1.61	<0.05	7.52 ± 3.19	4.74 ± 2.84	3.29 ± 1.94	<0.05
Usable blast. rate, %	**55.96%** (1436/2566)	**53.97%** (1011/1873)	**44.76%** (126/282)	0.0014	**44.79%** (474/1058)	**40.10%** (721/1798)	**40.06%** (247/617)	0.0342
Positive hCG, %	**79.89%** (139/174)	**78.29%** (119/152)	**85.71%** (30/35)	0.3250	**87.30%** (55/63)	**73.68%** (112/152)	**78.67%** (59/75)	0.1825
Biochemical PR, %	**12.64%** (22/174)	**12.50%** (19/152)	**20.00%** (7/35)	0.2476	**14.29%** (9/63)	**8.55%** (13/152)	**12.00%** (9/75)	0.2068
Spontaneous abortion rate, %	**5.17%** (9/174)	**3.29%** (5/152)	**2.86%** (1/35)	0.5582	**9.52%** (6/63)	**5.26** (8/152)	**9.33%** (7/75)	0.2491
Ongoing PR, %	**62.07%** 108/174	**62.50%** 95/152	**62.86%** 22/35	0.9301	**63.49%** (40/63)	**59.87%** (91/152)	**57.33%** (43/75)	0.4617
Live birth rate (2013–2015), %	**51.11%** (46/90)	**57.97%** (40/69)	**60.00%** (12/20)	0.4714	**60.00%** (27/45)	**53.85%** (49/91)	**54.75%** (29/53)	0.4965

are independent of the number of embryos generated (Ata *et al.*, 2012) or oocytes generated (Labarta *et al.*, 2017).

Our study presented evidence that euploidy rates are not affected by high gonadotropin dosages administered within recommended safety range (up to 450 IU per day) in the group of younger patients (under 38 y.o.). In the group of patient ≥38 y.o., we detected a gradual decline in euploidy rates when high dosages were used, even though it reached statistical significance only in a group of patients 38–40 y.o. It is unclear why the impact of high doses was evident only in the older patient population. We can speculate that it may be associated with the malfunction of intracellular reparation mechanisms and/or achromatic mitosis spindle assembly (Kuliev and Verlinsky, 2004; Vanneste *et al.*, 2009).

The study has several limitations. Due to the retrospective nature of the study, some key statistical parameters cannot be calculated. Possible bias of the embryo morphology assessment also can be limited in further studies by applying automated image recognition techniques and machine learning algorithms. Also, the patients who receive high gonadotropin doses are different from those who receive low doses. To control for this, it would be best to compare patients of similar age and ovarian reserve who received varying gonadotropin doses.

The here-presented results support those by Ng and Arce and collaborators, who reported no significant linear relationship between increasing dosages and embryo morphology (Ng *et al.*, 2000; Arce *et al.*, 2014). In our study, the proportion of good quality embryos decreased from 48.92% to 38.3%, while total dosage increased from under 3000 IU to over 5000 IU. This tendency was consistent among all age groups.

Baker and collaborators reported that gonadotropin dosage was negatively correlated with live birth rates (Baker *et al.*, 2015). While their work is based on analysis of a large number of IVF cycles in the USA, it important to note that these data were generated in non-PGS cycles, where fresh transfers were performed. Our data suggest that ongoing clinical pregnancy rates are not affected by high gonadotropin dosages administered within the dosage range recommended by the

manufacturer. Analysis of our findings also suggests that potential adverse consequences of high doses of exogenous gonadotropins on endometrium receptivity may be eliminated by avoiding fresh embryo transfer. Similar results were reported by Kol and collaborators (Kol *et al.*, 1999).

Conclusion

Our data provide support for the assumption that it is reasonable for physicians to use a relatively high gonadotropin dosage in IVF PGS cycles in order to retrieve an adequate number of oocytes.

Analysis of ongoing clinical pregnancy rates in different age groups demonstrated no negative effect of maternal age on pregnancy rates after single euploid embryo transfer. Using high gonadotropin dosages in the group of patients over 38 y.o. can significantly increase the quantity of aspirated oocytes while maintaining proper blastulation, euploidy and pregnancy rates. Euploidy and ongoing pregnancy rates are not affected by high gonadotropin dosage in IVF PGS cycles. Euploidy rates are primarily determined by patient's age regardless of the number of eggs retrieved.

To improve efficiency and cost-effectiveness in IVF PGS cycles, the maximum number of follicles recruited in each cycle should be limited only by medical safety concerns and patient well-being.

Authors' roles

Conceived and designed the study: O.O.B., M.D.H.; coordinated data collection: L.N.W., O.O.B.; analyzed the data: O.O.B., E.M.R., M.D.H.; drafted the manuscript: O.O.B, K.A.I., M.D.H., L.N.W., E.M.R. All authors interpreted the data.

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Conflict of interest

The authors declare that they have no conflict of interest

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