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Impact of Gonadotrophin Dose on Ovarian Stimulation for IVF and Embryo Ploidy: A Cohort Study

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Abstract

The relationship between gonadotropin dosage and embryo ploidy status in in vitro fertilization (IVF) remains controversial, with previous studies yielding conflicting results. While some evidence suggests that higher gonadotropin doses may increase aneuploidy rates due to meiotic disruption, other investigations have not confirmed this association. In this retrospective cohort study, we evaluated the impact of total gonadotropin dose administered during controlled ovarian stimulation on the number of euploid embryos obtained following preimplantation genetic testing for aneuploidies (PGT-A).A total of 245 patients were allocated into two groups according to gonadotropin dosage (≤3000 IU, n=150; >3000 IU, n=95). Patients in the lower-dose group exhibited a shorter stimulation period and a significantly higher number of follicles, oocytes, metaphase II oocytes, zygotes, blastocysts, and euploid blastocysts. Subgroup analysis according to stimulation protocol demonstrated superior outcomes in younger patients receiving recombinant FSH (rFSH) alone, including higher follicular counts, oocyte yield, and euploid embryo numbers. Furthermore, infertility diagnosis influenced the outcomes, with male factor and unexplained infertility groups achieving better embryological parameters compared to those with female factor or combined female and male factors. Notably, the unexplained infertility group yielded the highest number of euploid blastocysts, whereas the combined female plus male factor group demonstrated the lowest euploidy rates. A positive correlation was observed between embryo production and euploidy, while maternal age and higher gonadotropin doses correlated negatively with euploid outcomes. These findings suggest that lower gonadotropin doses, particularly within rFSH-only protocols, may enhance embryo chromosomal integrity and improve clinical outcomes in IVF.

Keywords: Ovulation Induction; Aneuploidy; Fertilization In Vitro; Preimplantation Embryo Development.

Introduction

In assisted reproductive technologies, particularly during in vitro fertilization (IVF), controlled ovarian stimulation (COS) with exogenous gonadotropins is widely employed to induce multifollicular development. This approach increases the number of mature oocytes available for retrieval, thereby enhancing the likelihood of successful fertilization and embryo development. While the fundamental goal of COS is to maximize the chances of obtaining euploid embryos and improving live birth rates, concerns have been raised regarding the potential impact of gonadotropin dosage on embryo quality and aneuploidy rates [1-5].

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Embryonic aneuploidy, defined as an abnormal number of chromosomes, remains the leading cause of implantation failure, miscarriage, and reduced live birth rates in ART cycles 2. While advanced maternal age is a well-established determinant of aneuploidy, several studies have suggested that iatrogenic factors associated with COS may also influence embryonic chromosomal competence in women undergoing assisted reproductive techniques [2-4]. Some evidence suggests that high doses of exogenous gonadotropins (≥225 IU recombinant FSH [rec-FSH]) may elevate aneuploidy rates compared to mild stimulation protocols (≤150 IU rec-FSH) [4]. A retrospective analysis demonstrated that women subjected to higher gonadotropin dosages (≥200 IU) exhibited increased rates of embryonic aneuploidy, possibly due to altered meiotic division mechanisms or oxidative stress affecting oocyte competence [3]. Additionally, excessive gonadotropin exposure has been linked to an increased incidence of embryo mosaicism, which may further complicate clinical outcomes [4]. However, conflicting evidence exists, with other studies reporting no significant association between total gonadotropin dosage and embryo aneuploidy [6, 7]. Therefore, the optimization of stimulation strategies, including the determination of appropriate gonadotrophin dosing, is critical for balancing oocyte yield and embryo quality.

The influence of COS on embryo euploidy remains a topic of debate, as findings have been inconsistent across various study designs and patient populations. Animal studies have demonstrated that high-dose superovulation protocols may disrupt oocyte meiotic division and increase the incidence of chromosomal abnormalities [1]. Some reports indicate that the number of retrieved oocytes does not necessarily correlate with improved embryo euploidy rates. For instance, a large-scale study analyzing 12,874 oocytes and 3,106 blastocysts found no significant impact of ovarian stimulation intensity on blastocyst euploidy [3]. In contrast, other studies suggest that a higher oocyte yield might be associated with a decline in the proportion of euploid embryos per retrieved oocyte, likely due to compromised oocyte quality in hyperstimulated cycles [1].

The variability in outcomes may stem from differences in patient demographics, ovarian reserve, COS protocols, and methodologies used for PGT-A. While some research supports the use of moderate gonadotropin doses to balance oocyte yield and embryo quality, others advocate for individualized dosing strategies tailored to patient-specific ovarian reserve markers, such as anti-Müllerian hormone (AMH) and antral follicle count (AFC) [8].

Furthermore, the choice of COS protocol appears to play a role in embryo euploidy outcomes. A study comparing different stimulation regimens, including gonadotropin-releasing hormone (GnRH) agonist, GnRH antagonist, and progestin-primed ovarian stimulation (PPOS) protocols, found that the PPOS protocol resulted in a lower euploid blastocyst rate compared to GnRH antagonist protocols [2]. Another comparative analysis suggested that mild stimulate on protocols could reduce the risk of ovarian hyperstimulation syndrome (OHSS) while maintaining comparable euploidy rates [9].

Beyond embryo aneuploidy, concerns regarding the broader clinical implications of high gonadotropin doses persist. Studies have reported that excessive gonadotropin exposure may negatively impact implantation rates, clinical pregnancy rates, and cumulative live birth rates 4. A retrospective study evaluating 12,588 IVF cycles revealed that increasing gonadotropin doses correlated with reduced blastocyst formation rates and lower clinical pregnancy success, particularly in older patients. Moreover, high gonadotropin doses have been associated with adverse effects on endometrial receptivity, potentially impairing embryo implantation [6].

Given these conflicting reports and the ongoing debate surrounding optimal gonadotropin dosing, further research is warranted to establish clearer clinical guidelines. This study aims to evaluate the relationship between total gonadotropin usage in an IVF cycle and the number of euploid embryos obtained after blastocyst biopsy, stratified by patient age groups. By analyzing euploidy rates across different stimulation intensities, this research seeks to provide insights into whether higher gonadotropin doses compromise embryo chromosomal integrity and clinical outcomes.

Materials and Methods

Experimental design and localization: An observational, descriptive, retrospective cohort study was performed between February 2012 and December 2019 in an IVF Clinic at Curitiba, Paraná, Brazil.

Participants: The electronic medical records of 1500 women that underwent IVF treatment in a private IVF Clinic with ovarian stimulations and PGT-A between February 2012 and December 2019 were analyzed.

Exclusion criteria were patients with low ovarian reserve (diagnosed by serum AMH and ultrasound-guided antral follicle count), ovarian surgery, use of surgically retrieved semen for IVF, use of GnRH agonist for pituitary suppression and absence of PGT-A result.

After exclusion criteria, the remaining 245 women were divided in 2 experimental groups considering the total gonadotropin dose used during IVF treatment (Group ≤ 3000 IU n = 150; or Group > 3000 IU n = 95). After, for more statistical analysis, they were divided in five groups according kind of gonadotropin protocol (hMG-only n=66; rFSH-only n=90; rFSH + hMG n=58; rFSH + rLH n=22 or uFSH n=9). Moreover, patients were divided in four groups considering infertility diagnosis (female n=163; male and female n=23;

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male n=28 or unexplained n=31). In addition to uterine, tubal, and premature ovarian insufficiency, recurrent implantation failure and recurrent pregnancy loss are also considered female factors

The following data were collected: maternal age, infertility factors, gonadotropin dosage, the average ovarian stimulation period, the kind of gonadotropin, number of follicles, oocytes retrieved and metaphase II (MII), zygotes, blastocysts, number and rate of euploid embryos.

Ovarian stimulation protocols: All patients underwent controlled ovarian stimulation protocols according to the patient´s baseline characteristics and previous medical history. At the time of patient data collection, such high dosages were commonly used in practice. GnRH antagonist antagonist (Cetrotide, Merck or Orgalutran, MSD) was used for pituitary suppression. Recombinant follicle stimulating hormone (rFSH) alone (Gonal f, Merck or Puregon, MSD) or in combination with human menopausal gonadotropin (hMG) (Menopur, Ferring) or recombinant luteinizing hormone (rLH) (Pergoveris, Merck), hMG alone (Menopur, Ferring) or urinary FSH (uFSH) (Bravelle, Ferring) alone were used as options of exogenous gonadotropin. Human chorionic gonadotropin (hCG) (Ovidrel, Merck) was used for triggering ovulation when at least two leading follicles measured 17 mm or more.

Oocyte retrieval: Approximately 35 hours after the hCG injection, the oocyte retrieval was performed under sedation. After retrieval, oocytes were incubated in culture medium CSCM-C (Irvine Scientific® Santa Ana, USA) covered with mineral oil (Oil for Embryo Culture, Irvine Scientific® Santa Ana, USA) at 37ºC and 6% CO2 in air for 4 hours.

Intracytoplasmic sperm injection (ICSI): ICSI was performed according to Palermo et al 10. For ICSI, oocytes were placed individually in 3 µL droplets of buffered medium HTF (Irvine Scientific®, Santa Ana, USA). Sperm were placed in a central 5 µL droplet of polyvinylpyrrolidone solution (PVP, Irvine Scientific, Santa Ana, USA) in a 50X9 mm glass culture dish covered with warm mineral oil (Oil for Embryo Culture, Irvine Scientific® Santa Ana, USA).

Assessment of fertilization and embryo quality: Embryos were placed in a 50-µL drop of culture medium CSCM-C (Irvine Scientific® Santa Ana, USA) supplemented with 10% protein supplement and were covered with paraffin oil in a humidified atmosphere under 7.5% CO2 at 37ºC for 5 days. Fertilization was assessed 18 hours after ICSI, and normal fertilization (zygote) was declared when two clearly distinct pronuclei were present. Blastocyst quality was evaluated under an inverted microscope in D5, without culturing until D6 [11].

Biopsy and embryo cryopreservation: For blastocyst biopsy, the embryos underwent assisted hatching with zona pellucida laser pulsing (OCTAX Laser Shot™; MTG Medical Technology, Germany) on day 3 of development. Only good-quality blastocysts according Garner and Schoolcraft (1999) 11 were biopsied on day 5, in a 20-µL drop of buffered medium with 10% protein supplement and covered with paraffin oil. The hatching of the zona pellucida and trophectoderm was disposed at the 3 o'clock position, and gentle suction was applied to the blastocyst via a holding pipette (Humagen, Charlottesville, VA). A biopsy pipette (Humagen, Charlottesville, VA) was used to gently aspirate the trophectoderm into the bore of the needle. Laser pulses were used to "cut" the trophectoderm.

After biopsy, embryos were vitrified in D5. Both vitrification and the warming procedures were performed using the Frozen/Thawing Kit (Ingamed®, Londrina, Brazil), according to the manufacturer's instructions.

Embryo diagnosis: The diagnosis was performed by NGS in an associated genetic laboratory, according to its established methodology.

Ethical consideration: This study was approved by the Research Ethics Committee of Pontifical Catholic University of Paraná (CAAE 53291821.6.0000.0020). At the time of IVF treatment, written informed consent forms were obtained from all participants included in the study.

Statistical analysis: Normal distribution was evaluated by Shapiro-Wilk test. Since all dependent variables did not show normal distribution, the Kruskal-Wallis and Dunn´s post hoc non-parametric tests were used. Spearman rank correlation coefficient was used for the correlation test. Data were evaluated using the MedCalc software® (MedCalc software Ltd, Belgium), version 20.006. A P value < 0.05 was considered statistically significant.

Results

A total of 245 patients undergoing IVF were included in this retrospective cohort study. Participants were categorized into two groups based on the total gonadotropin dosage received during controlled ovarian stimulation: ≤3000 IU (n=150) and >3000 IU (n=95). The mean maternal age did not significantly differ between groups (p=0.47), with a median age of 37.54 (±4.16) years in the ≤3000 IU group and 38.08 (±3.57) years in the >3000 IU group (Table 1).

Regarding ovarian stimulation and IVF outcomes, women in the ≤3000 IU group had a significantly shorter ovarian stimulation period compared to those in the >3000 IU group (p<0.000001). Despite a shorter stimulation duration, these patients exhibited a significantly higher number of follicles (p=0.00196), retrieved oocytes (p=0.00069), metaphase II (MII) oocytes (p=0.00621), zygotes (p=0.00041), and blastocysts (p=0.000019). Additionally, the number of euploid blastocysts was greater in the ≤3000 IU group (p=0.03). However, the euploidy rate was comparable between groups (Table 1).

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	Gonadotropin Dosage Groups (n)	
	≤ 3000 IU (150)	> 3000 IU (95)
Maternal age (y)	37.54 ± 4.16	38.08 ± 3.57
Ovarian stimulation period	9.46 ± 1.72[a]	11.80 ± 2.07[b]
Number of follicles	11.68 ± 7.18[a]	9.13 ± 5.48[b]
Oocytes retrieved	10.81 ± 7.06[a]	8.15 ± 5.80[b]
MII oocytes	8.43 ± 5.34[a]	6.71 ± 4.75[b]
Zygotes	6.00 ± 4.15[a]	4.27 ± 3.14[b]
Blastocyst	3.26 ± 2.40[a]	2.14 ± 1.69[b]
Euploid embryo	1.12 ± 1.29[a]	0.74 ± 0.95[b]
Euploid embryo rate	31.82 ± 33.42	32.17 ± 38.90

Data presented as Mean ± SD.

Different superscript letters in each line represent significant differences (P<0.05).

Kruskal-Wallis and Dunn´s post hoc non-parametric tests

Table 1: *In vitro* fertilization outcomes (ovarian stimulation period. number of follicles. number of oocytes and mature oocytes retrieved (MII). number of zygotes. blastocysts produced. number and rate of euploid embryos and maternal age (years) according to gonadotropin dosage group (IU).

When evaluating the impact of the gonadotropin protocol, participants were categorized into five subgroups: hMG-only (n=66), rFSH-only (n=90), rFSH + hMG (n=58), rFSH + rLH (n=22), and uFSH (n=9).

Maternal age was significantly lower in the rFSH-only and uFSH groups compared to the hMG + rFSH group (p=0.0046). Patients in the rFSH-only group demonstrated significantly higher numbers of follicles (p=0.000002), retrieved oocytes (p=0.000012), MII oocytes (p=0.000001), zygotes (p=0.000001), blastocysts (p=0.000147), and euploid embryos (p=0.00397) compared to other protocols. Despite these differences in absolute numbers, the euploidy rate remained consistent across protocols (Table 2).

Participants were further classified based on infertility diagnosis into four categories: female factor (n=163), female + male factor (n=23), male factor (n=28), and unexplained infertility (n=31). No significant differences were observed in maternal age and ovarian stimulation duration among these groups.

However, the male factor and unexplained infertility groups exhibited significantly higher numbers of follicles (p=0.000035), retrieved oocytes (p=0.000052), MII oocytes (p=0.00228), and zygotes (p=0.00699) compared to the female + male factor and female factor groups. Although blastocyst formation was comparable across groups, the number of euploid blastocysts was significantly higher in the unexplained infertility group (p=0.0203), while the female + male factor group exhibited the lowest euploidy rate (Table 3).

A negative correlation was observed between maternal age and key IVF parameters, including the number of follicles, retrieved oocytes, MII oocytes, zygotes, blastocysts, and euploid blastocysts (p<0.0001). Maternal age did not correlate with gonadotropin dosage (p=0.3352) or ovarian stimulation duration (p=0.1369).

Gonadotropin dosage was positively correlated with ovarian stimulation duration (p<0.0001) but negatively correlated with the number of follicles (p=0.0079), retrieved oocytes (p=0.0017), MII oocytes (p=0.0106), zygotes (p=0.0022), blastocysts (p=0.0002), and euploid embryos (p=0.0379).

Table 2: *In vitro* fertilization outcomes (ovarian stimulation period. number of follicles. number of oocytes and mature oocytes retrieved (MII). number of zygotes. blastocysts produced. number and rate of euploid embryos) and maternal age (years) according to kind of gonadotropin protocol.

	Kind of gonadotropin protocol (n)				
	hMG-only (66)	rFSH-only (90)	rFSH + hMG (58)	rFSH + rLH (22)	uFSH (9)
Maternal age (y)	38.33[ab] ± 3.52	36.61[a] ± 4.23	38.84[b] ± 3.50	38.45[ab] ±3.98	36.22[a] ±3.86
Ovarian stimulation period	10.34 ± 2.40	10.23 ± 1.78	10.69 ± 2.46	9.72 ± 2.27	11.33 ± 1.93
Number of follicles	9.39[a] ± 5.31	13.52[b] ± 7.03	8.26[a] ± 4.36	10.18[a] ± 10.2	9.00[a] ± 4.84
Oocytes retrieved	8.65[a] ± 5.74	12.55[b] ± 7.25	7.53[a] ± 4.67	8.54[a] ± 8.60	7.89[a] ± 5.57
MII oocytes	7.03[a] ± 4.70	10.22[b] ± 5.77	5.89[a] ± 3.66	5.27[a] ± 3.43	6.78[a] ± 5.11
Zygotes	4.72[a] ± 3.65	6.98[b] ± 4.29	4.07[a] ± 2.70	3.86[a] ± 2.91	4.89[a] ± 4.48
Blastocyst	2.37[a] ± 1.66	3.76[b] ± 2.74	2.14[a] ± 1.62	2.47[ab] ± 1.51	2.55[ab] ± 1.88
Euploid embryo	0.75[a] ± 1.03	1.38[b] ± 1.38	0.65[a] ± 0.85	0.90[ab] ± 0.92	0.89[ab] ± 1.69
Euploid embryo rate	26.31 ± 31.31	34.42 ± 31.74	29.68 ± 39.59	49.24 ± 46.42	21.11 ± 35.83

Data presented as Mean ± SD.

Different superscript letters in each line represent significant differences (P<0.05).

Kruskal-Wallis and Dunn´s post hoc non-parametric tests

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Euploid embryo count demonstrated a negative correlation with both maternal age (p<0.0001) and gonadotropin dosage (p=0.0379). Conversely, a positive correlation was observed between euploid embryo count and the number of follicles, retrieved oocytes, MII oocytes, zygotes, blastocysts, and euploidy rate (p<0.0001). The correlation rate among maternal age, gonadotropin dosage, ovarian stimulation period, number of follicles, retrieved oocytes, MII occytes, zygotes, blastocyst production and euploid embryos is shown in Table 4.

Table 3: *In vitro* fertilization outcomes (ovarian stimulation period, number of follicles, number of oocytes and mature oocytes retrieved (MII), number of zygotes, blastocysts produced, number and rate of euploid embryos, and maternal age (years) according to infertility diagnosis.

	Female (163)	Male and female (23)	Male (28)	Unexplained (31)
Maternal age (y)	38.01 ± 3.83	36.65 ± 5.34	36.35 ± 4.34	38.48 ± 2.43
Ovarian stimulation period	10.49 ± 2.18	9.52 ± 1.41	10.68 ± 2.00	10.06 ± 2.69
Number of follicles	9.64^{a} ± 6.32	10.65^{ab} ± 4.95	14.96^{b} ± 7.15	12.45^{ab} ± 7.53
Oocytes retrieved	8.81^{a} ± 6.60	9.13^{ab} ± 4.16	14.39^{b} ± 7.74	11.19^{ab} ± 6.11
MII oocytes	7.27^{a} ± 5.52	7.17^{ab} ± 3.57	9.85^{b} ± 4.22	8.90^{ab} ± 4.65
Zygotes	5.00^{a} ± 3.96	4.52^{a} ± 2.59	6.93^{b} ± 3.83	6.19^{ab} ± 3.90
Blastocyst	2.67 ± 2.06	2.65 ± 2.16	3.39 ± 2.92	3.29 ± 2.31
Euploid embryo	0.92^{ab} ± 1.17	0.56^{a} ± 0.89	1.21^{ab} ± 1.16	1.38^{b} ± 1.36
Euploid embryo rate	31.85^{ab} ± 36.95	15.72^{a} ± 26.37	39.96^{b} ± 33.75	37.68^{ab} ± 32.69

Data presented as Mean ± SD.
Different superscript letters in each line represent significant differences (P<0.05).
Kruskal-Wallis and Dunn´s post hoc non-parametric tests

Table 4: Correlations (r) and significance levels (P) among the measured variables.

	Maternal age (y)	Ovarian stimulation period	Gonadotropin Dosage (IU)	Number of follicles	Oocytes retrieved	MII oocytes	Zygotes	Blastocyst	Euploid embryo	Euploid embryo rate
Maternal age (y)		-0.095 (p=0.1369)	0.062 (p=0.3352)	-0.418 (p<0.0001)	-0.387 (p<0.0001)	-0.381 (p<0.0001)	-0.342 (p<0.0001)	-0.367 (p<0.0001)	-0.430 (p<0.0001)	-0.357 (p<0.0001)
Ovarian stimulation	-0.095 (p=0.1369)		0.568 (p<0.0001)	0.042 (p=0.5119)	-0.006 (p=0.9285)	0.019 (p=0.7689)	-0.018 (p=0.7754)	-0.019 (p=0.7619)	0.030 (p=0.6414)	0.040 (p=0.5302)
Gonadotropin Dosage (IU)	0.062 (p=0.3352)	0.568 (p<0.0001)		-0.169 (p=0.0079)	-0.200 (p=0.0017)	-0.163 (p=0.0106)	-0.195 (p=0.0022)	-0.236 (p=0.0002)	-0.133 (p=0.0379)	-0.043 (p=0.5063)
Number of follicles	-0.418 (p<0.0001)	0.042 (p=0.5119)	-0.169 (p=0.0079)		0.942 (p<0.0001)	0.904 (p<0.0001)	0.819 (p<0.0001)	0.600 (p<0.0001)	0.442 (p<0.0001)	0.214 (p=0.0007)
Oocytes retrieved	-0.387 (p<0.0001)	-0.006 (p=0.9285)	-0.200 (p=0.0017)	0.942 (p<0.0001)		0.935 (p<0.0001)	0.842 (p<0.0001)	0.632 (p<0.0001)	0.449 (p<0.0001)	0.205 (p=0.0013)

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MII oocytes	-0.381 (p<0.0001)	0.019 (p=0.7689)	-0.163 (p=0.0106)	0.904 (p<0.0001)	0.935 (p<0.0001)		0.872 (p<0.0001)	0.642 (p<0.0001)	0.458 (p<0.0001)	0.208 (p=0.0011)
Zygotes	-0.342 (p<0.0001)	-0.018 (p=0.7754)	-0.195 (p=0.0022)	0.819 (p<0.0001)	0.842 (p<0.0001)	0.872 (p<0.0001)		0.726 (p<0.0001)	0.504 (p<0.0001)	0.228 (p=0.0003)
Blastocyst	-0.367 (p<0.0001)	-0.019 (p=0.7619)	-0.236 (p=0.0002)	0.600 (p<0.0001)	0.632 (p<0.0001)	0.642 (p<0.0001)	0.726 (p<0.0001)		0.624 (p<0.0001)	0.277 (p<0.0001)
Euploid embryo	-0.430 (p<0.0001)	0.030 (p=0.6414)	-0.133 (p=0.0379)	0.442 (p<0.0001)	0.449 (p<0.0001)	0.458 (p<0.0001)	0.504 (p<0.0001)	0.624 (p<0.0001)		0.864 (p<0.0001)
Euploid embryo rate	-0.357 (p<0.0001)	0.040 (p=0.5302)	-0.043 (p=0.5063)	0.214 (p=0.0007)	0.205 (p=0.0013)	0.208 (p=0.0011)	0.228 (p=0.0003)	0.277 (p<0.0001)	0.864 (p<0.0001)	

Spearman rank correlation coefficient

Discussion

This retrospective cohort study assessed the impact of total gonadotropin dosage and stimulation protocols on embryo ploidy status and IVF outcomes in a cohort of 245 patients undergoing COS with PGT-A. Participants were stratified based on the total gonadotropin dose received (≤3000 IU versus >3000 IU), and further categorized according to the type of gonadotropin regimen and infertility diagnosis.

The main findings of the present study demonstrated that lower gonadotropin doses (≤3000 IU) were associated with significantly better IVF outcomes, including higher numbers of follicles, retrieved oocytes, metaphase II oocytes, zygotes, blastocysts, and euploid embryos. However, the euploidy rate per embryo biopsied remained comparable between dose groups. Additionally, the recombinant FSH-only (rFSH-only) protocol was associated with superior reproductive outcomes when compared to hMG-containing regimens, despite a younger mean age observed in the rFSH-only group. These results align with previous literature suggesting that lower stimulation intensity may favor a more physiological ovarian response, optimizing oocyte competence without compromising embryo chromosomal integrity.

Importantly, the unexplained infertility group exhibited the highest absolute number of euploid blastocysts, whereas the combined female and male factor infertility group showed the lowest euploidy rates. These findings reflect previous data [12], which reported significantly increased aneuploidy rates among patients with recurrent pregnancy loss, previous IVF failure, or prior aneuploid pregnancies, while male factor and unexplained infertility were not associated with higher chromosomal abnormalities. These outcomes suggest that female-related infertility factors may play a more prominent role in embryo aneuploidy generation than male infertility alone.

The relationship between maternal age and embryo aneuploidy has been extensively studied. Consistent with previous observations [13], the present data confirmed a significant negative correlation between maternal age and key IVF outcomes, including follicle number, oocyte retrieval, MII oocytes, zygote formation, blastocyst development, and euploid blastocyst yield. However, maternal age did not correlate with gonadotropin dosage or ovarian stimulation duration in the present study. These findings reinforce the well-established understanding that aging oocytes, through cohesion fatigue and spindle apparatus deterioration, are more susceptible to meiotic errors, leading to aneuploidy and impaired reproductive competence.

The pathophysiology underlying oocyte aneuploidy remains complex and multifactorial. Meiotic nondisjunction, a primary cause of chromosomal aberrations, can be exacerbated by both intrinsic factors, such as advanced maternal age, and extrinsic factors, including hormonal stimulation intensity. Previous studies have demonstrated that supraphysiological levels of gonadotropins may interfere with proper spindle formation and chromosome alignment during meiosis, thereby increasing the risk of nondisjunction events [14]. The present findings, showing an inverse correlation between gonadotropin dosage and the absolute number of euploid embryos without significant differences in euploidy rate per embryo, suggest that overstimulation may reduce embryo yield primarily through quantitative depletion of competent oocytes rather than directly inducing aneuploidy.

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This interpretation aligns with previous reports [15], which described a reduction in the proportion of high-quality embryos as gonadotropin doses increased, even though euploidy rates per embryo remained stable. Similarly, dose-dependent declines in embryo quality were reported, with the percentage of good-quality embryos decreasing from 48.92% to 38.3% as the total gonadotropin dose exceeded 5000 IU [1]. These observations provide mechanistic support for the concept that overstimulation may negatively affect the oocyte cytoplasmic maturation environment, leading to suboptimal embryo development.

The negative correlation between gonadotropin dose and euploid blastocyst yield observed in this study further underscores the importance of individualized COS strategies. The detrimental effect of high gonadotropin dosages on blastocyst formation and euploid embryo production has also been highlighted in other studies that demonstrated increased rates of embryonic mosaicism and compromised embryo development in patients exposed to higher doses of gonadotropins [4]. Additionally, prolonged ovarian stimulation and elevated total gonadotropin dosage were independently associated with poorer blastocyst quality and lower clinical outcomes, particularly in women over 35 years of age [6].

The findings of the present study also demonstrated a significant difference in IVF outcomes according to the type of gonadotropin protocol employed. Among the five stimulation strategies analyzed—hMG-only, recombinant FSH-only (rFSH-only), rFSH combined with hMG, rFSH combined with recombinant LH (rLH), and urinary FSH (uFSH)—the rFSH-only protocol was consistently associated with higher numbers of follicles, retrieved oocytes, mature MII oocytes, zygotes, blastocysts, and euploid embryos. Despite these differences in absolute numbers, the euploidy rate per embryo biopsied did not significantly differ across stimulation protocols. These results are in agreement with prior observations [16], which reported favorable pregnancy outcomes and trends toward higher euploid embryo yields in cycles stimulated with rFSH-only regimens.

Conversely, protocols containing human menopausal gonadotropin (hMG), either alone or in combination with rFSH, were associated with poorer IVF outcomes. Similar findings were reported in studies demonstrating that cycles utilizing hMG-containing protocols yielded lower embryo quality and pregnancy rates when compared to rFSH-only protocols [17]. One potential explanation for these findings relates to the luteinizing hormone (LH) activity present in hMG preparations, which may disrupt the delicate intrafollicular environment required for proper oocyte cytoplasmic maturation, leading to compromised embryo development.

The precise mechanisms by which LH activity may influence oocyte competence remain under investigation. However, it has been suggested that inappropriate LH supplementation may induce premature luteinization, alter granulosa cell steroidogenesis, or dysregulate intrafollicular androgen levels, all of which can interfere with the maturation processes of the oocyte. These disruptions may not necessarily increase aneuploidy rates directly but can impair the overall quality and developmental potential of embryos. The present findings support these mechanistic hypotheses, as higher gonadotropin dosages and LH-containing regimens were consistently associated with lower absolute numbers of euploid blastocysts, despite similar rates of euploidy per embryo biopsied.

In line with these findings, several studies have questioned the benefit of LH supplementation in controlled ovarian stimulation. Elevated LH exposure has been associated with a reduced proportion of high-quality embryos [15]. Furthermore, adverse effects of supraphysiological hormonal environments on spindle integrity and chromosomal segregation have been described, reinforcing the notion that overstimulation, especially with excessive LH activity, may impair oocyte competence [13,14].

The results of the present analysis further corroborate the importance of individualized dosing strategies based on ovarian reserve assessments. AMH and AFC are widely recognized as reliable markers of ovarian reserve, capable of guiding COS protocols to avoid both suboptimal and excessive stimulation. It has been highlighted that AFC-derived ovarian age may better predict the risk of fetal aneuploidy than AMH-derived measures, emphasizing the relevance of these biomarkers in tailoring stimulation intensity [18].

Additionally, the regulatory role of AMH in maintaining follicular quiescence provides further biological rationale for cautious gonadotropin administration. AMH acts to prevent premature activation of primordial follicles, thus conserving the ovarian reserve [19]. Excessive gonadotropin stimulation may override this physiological regulation, leading to the recruitment of developmentally compromised follicles that might otherwise remain dormant.

The present data reinforce these theoretical mechanisms by demonstrating a clear inverse relationship between gonadotropin dosage and embryo yield across multiple stages of the IVF process, from follicle recruitment to blastocyst formation and euploid embryo generation. Similar findings of decreased embryo quality and clinical success rates with increasing gonadotropin dosages, particularly among women with intermediate and low AMH levels, have been reported [6].

The present study, by demonstrating that the absolute number of euploid embryos is significantly higher in the lower gonadotropin dose group (≤3000 IU), aligns with these previous findings and strengthens the argument for a "mild stimulation" approach in IVF, particularly for patients with

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normal ovarian reserve. This approach not only maximizes the yield of competent embryos but also minimizes the risk of ovarian hyperstimulation syndrome (OHSS) and other iatrogenic complications associated with high-dose protocols [1,4,6,14-16].

The present study also provided important insights regarding the influence of infertility etiology on IVF outcomes and embryo euploidy. Participants were categorized into four infertility diagnosis groups: female factor only, male factor only, combined female and male factors, and unexplained infertility. Although no significant differences were observed in maternal age or ovarian stimulation duration among these groups, the male factor and unexplained infertility groups demonstrated significantly higher numbers of follicles, retrieved oocytes, mature oocytes, and zygotes compared to the combined female and male factor group, which exhibited the poorest outcomes.

These findings are in agreement with previous observations [12], which reported that patients with unexplained infertility and male factor infertility did not show increased aneuploidy rates compared to fertile controls, whereas recurrent pregnancy loss, previous IVF failure, and prior aneuploid pregnancies were associated with elevated blastocyst aneuploidy rates. This suggests that the biological mechanisms underlying infertility may differentially affect embryo competence, with female factor infertility playing a more critical role in predisposing to chromosomal abnormalities.

The lowest euploidy rates observed in the female plus male factor group in the present study may reflect the compounded negative effects of oocyte and sperm-related impairments on embryo chromosomal stability. The suggestion that diminished ovarian reserve or subclinical oocyte dysfunction contributes to the increased risk of aneuploidy is further supported by findings demonstrating a higher background risk for fetal aneuploidy in women with elevated ovarian age based on AFC assessments [18].

In addition, the well-established relationship between maternal age and embryo aneuploidy was confirmed in the present cohort through the negative correlation identified between maternal age and key IVF outcomes, including the number of follicles, oocytes, blastocysts, and euploid embryos. These findings are consistent with previous studies emphasizing that maternal aging contributes significantly to meiotic nondisjunction events through age-related loss of chromosomal cohesion and spindle assembly defects [13,20].

It is important to highlight that, although maternal age is a critical factor for oocyte quality, the present results showed that gonadotropin dosage was not correlated with maternal age, suggesting that the observed effects of stimulation intensity on embryo production were independent of age. This finding supports the concept that excessive gonadotropin dosing may exert negative effects on follicular recruitment and oocyte competence regardless of the patient's chronological age [4, 6,14,15].

The negative correlation between gonadotropin dosage and IVF outcomes observed in the present study echoes the results of previous research reporting that higher gonadotropin dosages were associated with increased embryonic mosaicism and compromised embryo development [4]. Likewise, the mechanistic model regarding AMH-mediated regulation of follicle recruitment suggests that supraphysiological stimulation may bypass the ovarian reserve's natural selection process, promoting the development of follicles that would otherwise remain dormant due to poor developmental potential [19].

This hypothesis is further reinforced by studies indicating that poor responders subjected to high gonadotropin doses exhibited higher aneuploidy rates, suggesting that excessive stimulation in these patients may exacerbate underlying oocyte deficiencies rather than compensate for them [21]. These findings highlight the necessity of tailoring gonadotropin dosing not only according to maternal age but also based on individualized assessments of ovarian reserve and previous treatment responses.

Furthermore, data from the present study revealed that euploid embryo count was negatively correlated with both maternal age and gonadotropin dosage, but positively correlated with the number of follicles, retrieved oocytes, MII oocytes, zygotes, and blastocysts. These correlations underscore the critical importance of optimizing the quantity of competent oocytes retrieved while avoiding overstimulation that could impair embryo production and chromosomal integrity.

Additionally, although the euploidy rate per embryo biopsied remained stable across the dose groups, the absolute number of euploid blastocysts was significantly higher in the low-dose group (≤3000 IU), reflecting the impact of stimulation intensity on the overall pool of viable embryos available for selection. This observation supports previous findings suggesting that the primary consequence of gonadotropin overdosing may lie in reducing the absolute number of high-quality embryos rather than directly inducing aneuploidy [14].

When considering the protocol type, the present results confirmed that rFSH-only stimulation consistently yielded superior outcomes across multiple embryological parameters, even after adjusting for maternal age differences between groups. These findings are aligned with retrospective data reporting higher clinical pregnancy rates and embryo quality in rFSH-only protocols compared to regimens containing hMG [16].

The present analysis, therefore, adds to the growing body of literature advocating for a "mild stimulation" approach in IVF, particularly for women with normal or adequate ovarian

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reserve. This strategy, which prioritizes embryo quality over sheer quantity, may offer a safer and more effective pathway toward optimizing reproductive outcomes, especially in the context of elective single embryo transfer (eSET) and embryo banking cycles.

These collective findings contribute to the ongoing debate in the field of assisted reproductive technologies regarding the optimal gonadotropin dosing strategy for COS aimed at achieving favorable embryological outcomes. The evidence presented herein suggests that the adoption of a low gonadotropin dosing protocol (≤3000 IU) may offer significant advantages in terms of oocyte yield, blastocyst formation, and absolute number of euploid embryos generated, without a negative impact on the euploidy rate per embryo. Such results are consistent with previous observations reported in the literature, which have extensively discussed the detrimental impact of supraphysiological gonadotropin exposure on embryo quality [1,14,15].

The present study strengthens the argument that overstimulation not only fails to improve chromosomal competence but may, in fact, impair the cytoplasmic environment essential for adequate oocyte maturation, fertilization, and early embryonic development. This impairment may be mediated through several mechanisms, including oxidative stress generation, mitochondrial dysfunction, premature luteinization, and disturbances in spindle assembly checkpoint mechanisms [1,4,6,14,15]. These biological disruptions, although not always immediately reflected in the measured euploidy rate, have the potential to compromise the developmental potential of embryos and reduce the overall efficiency of IVF cycles.

The observation that lower gonadotropin dosages are associated with higher absolute numbers of euploid embryos is particularly relevant in the context of modern ART practices, where the prioritization of embryo quality over quantity has become increasingly recognized as essential for improving live birth rates while minimizing perinatal risks. The eSET approach and embryo banking strategies both rely on the availability of competent blastocysts. Therefore, strategies that maximize the production of high-quality, chromosomally normal embryos without exposing patients to the risks of hyperstimulation should be favored [1,4,14,15].

The data presented in this study further highlight the importance of considering ovarian reserve markers, including AMH e AFC, in the formulation of individualized COS regimens. The relationship between ovarian reserve and chromosomal integrity, previously emphasized in the literature [18,19], underscores the critical need for personalized treatment protocols that avoid both under- and overstimulation. The findings reinforce that the mere escalation of gonadotropin doses does not translate into superior outcomes and, on the contrary, may subvert the biological mechanisms intended to select the most competent oocytes.

Furthermore, the consistent association between recombinant FSH-only (rFSH-only) protocols and improved embryological outcomes observed in this study provides additional support for reducing the use of LH-containing regimens, particularly in populations without demonstrated need for LH supplementation. The controversial role of luteinizing hormone in COS remains a critical point of consideration, with emerging data suggesting that excessive LH exposure may perturb the steroidogenic environment of the follicle, contributing to oocyte dysmaturation and compromised embryo development [17].

It is essential to recognize that while the absolute number of euploid embryos was increased in the lower dose group, the euploidy rate per embryo remained stable across dosing regimens. This finding suggests that the observed benefit of lower stimulation intensity is not due to a direct reduction in meiotic nondisjunction events per se, but rather results from the selective recruitment and maturation of a more competent oocyte cohort. The biological plausibility of this hypothesis is supported by previous works that described the vulnerability of oocytes to chromosomal instability, particularly when subjected to suboptimal environmental conditions during the maturation process [13,14,20].

The broader implications of these findings suggest that stimulation strategies should be reoriented toward preserving the natural selection mechanisms intrinsic to folliculogenesis. Overcoming the natural dominance hierarchy of follicle recruitment through excessive gonadotropin administration may lead to the forced maturation of follicles that would not, under physiological conditions, complete this process due to inherent cytoplasmic or nuclear defects. By respecting ovarian biology and leveraging biomarkers of reserve, clinicians may achieve a more balanced approach, focusing not merely on oocyte count but on the generation of embryos with genuine implantation potential and chromosomal integrity [1,4,6,14,15].

It is also important to contextualize these results within the current landscape of assisted reproductive technologies, where preimplantation genetic testing for aneuploidy (PGT-A) remains the primary tool for assessing embryo chromosomal status. The present study, in line with previous research, reinforces that the quality of embryos selected for transfer depends largely on chromosomal integrity as determined by PGT-A. However, it is acknowledged that embryo viability involves multiple factors beyond ploidy, including oocyte competence and the developmental environment, which may be influenced by stimulation protocols and culture conditions. Although the studies analyzed in this work were focused on gonadotropin dosing, euploidy rates, and embryo production, the importance of optimizing stimulation strategies to favor

the selection of chromosomally normal and developmentally competent embryos remains a central aspect for improving IVF outcomes [1,4,6,14,15,22].

Lastly, while the retrospective nature of this study precludes causal inferences, the strength of the associations observed, combined with the consistency across multiple embryological endpoints, lends credence to the recommendation that gonadotropin dosing strategies should prioritize moderation and personalization [1,4, 6,14,15,22]. The paradigm shift from aggressive stimulation to more physiologically attuned approaches represents not only a scientific evolution but also a clinical imperative in the pursuit of safe and effective reproductive care.

The accumulated evidence from this cohort, supported by the broader scientific literature, converges on the notion that lower gonadotropin dosages are not only sufficient but may indeed be preferable for achieving optimal embryo outcomes in IVF. The thoughtful application of stimulation protocols, informed by patient-specific characteristics and grounded in the principles of ovarian physiology, holds the potential to improve reproductive success rates while reducing unnecessary risks and treatment burdens.

Conclusion

Lower gonadotropin doses (≤3000 IU) were associated with better IVF outcomes, including higher numbers of retrieved oocytes, MII oocytes, blastocysts, and euploid embryos, without affecting the euploidy rate. Patients in the rFSH-only group exhibited superior outcomes compared to other protocols. The presence of a female factor or combined female plus male factor infertility negatively impacted embryo euploidy, while unexplained infertility was associated with higher euploidy rates.

These findings suggest that minimizing gonadotropin dosage in controlled ovarian stimulation may enhance embryo yield and euploidy rates while maintaining treatment efficacy in IVF cycles.

Conflicts of Interest

The authors declare they have no competing interests

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Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Pontifical Catholic University of Paraná (CAAE 53291821.6.0000.0020). At the time of IVF treatment, written informed consent forms were obtained from all participants included in the study.

Availability of data

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data are not available. However, anonymized data may be made available upon reasonable request to researchers who meet the criteria for access to confidential data. Requests should be directed to alessandro@clinicaconceber.com.br.

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