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Flexible progestin-primed ovarian stimulation is a safe and effective alternative to GnRH antagonist protocol in PGT-A cycles

Esnek oral progestin destekli over stimülasyonu protokolü PGT-A sikluslarında gonadotropin salgılatıcı hormon antagonisti protokolüne güvenli ve etkili bir alternatiftir

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Abstract

Objective: To test the hypothesis that flexible progestin-primed ovarian stimulation (fPPOS) is non-inferior to the gonadotropin-releasing hormone (GnRH) antagonist protocol in terms of safety and efficacy for patients undergoing controlled ovarian hyperstimulation and preimplantation genetic testing for aneuploidy (PGT-A).

Materials and Methods: This retrospective analysis included data from 548 cycles involving 367 women aged 35 to 45 years. The fPPOS and GnRH antagonist groups comprised 307 cycles (56%) and 241 cycles (44%), respectively. All participants underwent absolute blastocyst culture, trophectoderm biopsy, and PGT-A, with advanced maternal age as the sole indication. The primary outcomes were incidence of premature luteinizing hormone (LH) rise (>10 mIU/mL), cycle cancellation due to premature ovulation, and euploid blastocyst rate per injected metaphase II oocyte. Spearman's rho correlation and the generalized linear model (logit) were applied for statistical analysis.

Results: The incidence of premature LH rise (8.2% versus 6.6%; p=0.302) and cycle cancellation due to premature ovulation (2% versus 0.4%; p=0.112) did not differ significantly between the fPPOS and GnRH antagonist groups. Maturation, fertilization, and blastulation rates were also similar (p>0.05). The euploid blastocyst rates per biopsy (50.12% versus 53.06%; p=0.317) and per injected metaphase II oocyte (23.84% versus 23.34%; p=0.231) were comparable between groups. Secondary outcomes, including rates of positive pregnancy tests, implantation, ongoing pregnancy, biochemical pregnancy losses, and early miscarriages, were also similar (p>0.05).

Conclusion: The fPPOS protocol represents a viable alternative to the GnRH antagonist protocol for patients aged 35 years or older undergoing PGT-A.

Keywords: Progestin-primed ovarian stimulation, gonadotropin-releasing hormone antagonist, controlled ovarian hyperstimulation, preimplantation genetic testing for aneuploidy

PRECIS: The flexible administration of medroxyprogesterone acetate effectively prevents the luteinizing hormone rise during ovarian hyperstimulation when compared to gonadotropin-releasing hormone antagonists. Furthermore, it does not have an adverse effect on euploidy rates.

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Öz

Amaç: Çalışmanın amacı, kontrollü over hiperstimülasyonunda esnek oral progestin destekli (eOPD) protokolün güvenliğini ve etkinliğini değerlendirmek ve aneuploidi için preimplantasyon genetik testi (PGT-A) dahil olmak üzere embriyolojik süreçler üzerindeki etkisini araştırmaktır.

Gereç ve Yöntemler: Bu çalışma, 35-45 yaş aralığındaki 367 kadına ait 548 tedavi siklusundan elde edilen verilerin retrospektif analizini içermektedir. eOPD grubunda 307 siklus (%56) ve gonadotropin salgılayıcı hormon (GnRH) antagonisti grubunda 241 siklus (%44) yer almaktaydı. Mutlak blastokist kültürü uygulandı. İleri anne yaşı endikasyonu ile trofektoderm biyopsisi ve PGT-A yapıldı. Birincil sonuçlar arasında erken luteinize edici hormon (LH) yükselme oranları (>10 mIU/mL), erken ovülasyona bağlı siklus iptalleri ve enjekte edilen metastaz II (MII) oosit başına öploid blastokist oranı yer alıyordu. Spearman'ın rho korelasyonu ve genelleştirilmiş doğrusal model (logit) kullanıldı.

Bulgular: eOPD grubundaki erken LH yükselişinin (%8,2-6,6; p=0,302) ve erken ovulasyona bağlı siklus iptalinin (%2-0,4; p=0,112) insidansı GnRH antagonisti grubundakine benzerdi. Olgunlaşma, döllenme ve blastülasyon oranları benzerdi (p>0,05). Biyopsi yapılan blastokist başına (%50,12-53,06; p=0,317) ve enjekte edilen MII oosit başına (%23,84-23,34; p=0,231) elde edilen öploid blastokist oranı eOPD ve GnRH antagonisti grupları arasında benzerdi. Pozitif gebelik testi oranları, implantasyon, devam eden gebelik, biyokimyasal gebelik kayıpları ve erken düşükler gibi ikincil sonuçlar gruplar arasında benzerdi (p>0,05).

Sonuç: eOPD protokolü, PGT-A uygulanan 35 yaş üstü hastalarda GnRH antagonist protokolüne uygulanabilir bir alternatif sunmaktadır.

Anahtar Kelimeler: Oral progestin destekli protokol, gonadotropin salgılatıcı hormon antagonisti, kontrollü overyan hiperstimülasyon, preimplantasyon genetik tanı

Introduction

Controlled ovarian hyperstimulation (COH) aims to enhance the quantity of oocytes retrieved and, consequently, the number of embryos generated. Follicles that would typically undergo atresia under natural circumstances are stimulated to grow by administration of gonadotropins, thereby increasing the couple's probability of achieving conception. It is important to note that not every oocyte collected has the same potential for developing into a viable embryo[1].

A fundamental aspect of COH is preventing premature luteinizing hormone (LH) surges[2]. The primary pharmacological agents employed for this purpose have long been gonadotropin-releasing hormone (GnRH) agonists and antagonists. In the literature, a reported incidence of premature LH surge ranges from 0.34% to 8% in the context of treatments involving GnRH antagonists[3]. The incidence is comparable in the short agonist protocol[4] but significantly lower in the long GnRH agonist protocol[5,6]. The primary disadvantages of these agents include their mode of administration, which requires injection, and the resulting high treatment costs[7].

Since the beginning of the last decade, progestin-primed ovarian stimulation (PPOS) has become one of the most frequently used alternatives to prevent a premature LH surge in in vitro fertilization (IVF) treatments. Research suggests that elevated serum endogenous progesterone levels during the luteal phase of COH can sufficiently inhibit pituitary function, indicating that additional GnRH antagonist administration may not be necessary[8]. This concept served as the foundation for the PPOS strategy. A landmark randomized controlled trial (RCT) published in 2015 demonstrated that, among normoresponder patients, the rates of premature LH surges, the developmental potential of the embryos, and pregnancy outcomes in the medroxyprogesterone acetate (MPA) group were similar to those in the GnRH antagonist group[9]. The primary drawback of this protocol is that fresh embryo transfer (ET) cannot be executed, as the endometrium is exposed to exogenous progesterone prematurely during the follicular phase, unlike with GnRH preparations[10]. Considering this perspective, it would be prudent to analyze its impact on embryological outcomes and pregnancy rates in protocols involving freeze-all strategies, such as oocyte cryopreservation, preimplantation genetic testing, embryo cryobanking, and prevention of ovarian hyperstimulation syndrome[11].

Research has consistently demonstrated that the aneuploidy risk in embryos increases with advancing maternal age (AMA)[12]. This relationship has been validated through both preimplantation genetic testing for aneuploidy (PGT-A)[13] and analyses of products of conception[14]. Utilizing next-generation sequencing (NGS) to evaluate trophectoderm (TE) biopsy samples during the PGT-A process can provide significant benefits for patients in this demographic, owing to its high negative predictive value[15]. Today, the predominant practice for PGT-A cycles globally involves TE biopsy followed by the vitrification of blastocysts. The increased embryo survival rates resulting from the vitrification method have led to a considerable increase in the utilization of frozen ET applications in recent years[16].

Promising outcomes for various oral progestin regimens have been demonstrated in IVF/intracytoplasmic sperm injection (ICSI) treatments during the past decade[17]. However, studies evaluating flexible protocols in which exogenous progesterone is introduced later in the stimulation course remain limited compared with conventional approaches. This disparity may indicate ongoing clinical caution regarding flexible protocols for LH surge management. Therefore, this study compared the effects of the flexible PPOS (fPPOS) protocol, which used MPA as the oral progestin, with those of the GnRH antagonist protocol, focusing on the stimulation period and embryological outcomes in PGT-A cycles. This study is the first extensive analysis directly comparing these

protocols, using a dataset comprising more than 500 cycles in this specific context.

Materials and Methods

Study Design

The medical records of participants who underwent ICSI with PGT-A from June 1, 2020, to December 31, 2024, were retrieved from the internal medical database. The Institutional Review Board of Acıbadem Hospital (date: 02.01.2025) approved the research. In this study, informed consent was not considered necessary because the data used were entirely anonymized. All patient identifiers were diligently removed prior to analysis to ensure that confidentiality standards were maintained. This study was conducted in accordance with the ethical guidelines set forth in the revised Declaration of Helsinki (2020). The fourth author, H.B., served as the embryologist for patients involved in this study.

A total of 894 treatment cycles involving 590 women were included in this analysis. From this cohort, a specific subset of 346 treatment cycles involving 223 women was excluded based on the following criteria: i) presence of monogenic disorders in either the maternal or paternal lineage and identification of structural chromosomal abnormalities; ii) history of recurrent miscarriages or repeated implantation failures; iii) body mass index (BMI) below 18 kg/m² or above 35 kg/m²; iv) male participants with a sperm concentration of less than 2 million or those requiring testicular sperm aspiration or percutaneous epididymal sperm aspiration. In accordance with the legal regulations in our country, the use of donor gametes is not permitted. Additionally, all procedures were carried out exclusively with fresh gamete cells and embryos.

The study included PGT-A procedures that were performed solely on couples classified as AMA. The definition of AMA aligns with the most recent recommendations set forth by the European Society of Human Reproduction and Embryology PGT Consortium, which establishes an age threshold of 35 years or older[18]. The upper age limit was established at 45 years.

A total of 548 treatment cycles involving 367 women were included in the final analysis. Of these, 307 cycles (56%) were assigned to the fPPOS group and 241 cycles (44%) to the GnRH antagonist group. Absolute blastocyst culture was performed as a standard procedure for all patients scheduled for PGT-A.

Controlled Ovarian Hyperstimulation

COH was initiated using either the fPPOS or GnRH antagonist protocol. The initial dosage was tailored by considering the woman's age, BMI, initial follicle sizes, and prior treatment response. Recombinant follitropin alpha follicle-stimulating hormone (r-FSH) (Gonal F; Merck Serono S.p.A., Modugno, Italy) or menotropin (HP-hMG) (Meriofert; IBSA, Switzerland) were administered either individually or in combination. The analysis did not encompass mild stimulation protocols that exclusively employed clomiphene citrate or letrozole. The ovarian response was assessed through a series of transvaginal scans and hormonal monitoring. Serum LH levels were monitored systematically in both groups from the initiation of LH suppression until the day of ovulation triggering. Any necessary dosage adjustments were made at the attending clinicians' discretion based on the observed ovarian response. In the fPPOS protocol, 5 mg of MPA (Tarlusal; Deva, Türkiye) was administered twice daily, once the follicles reached 12 mm in size, and the treatment continued until the ovulation trigger day. In the GnRH antagonist protocol, 0.25 mg/day cetrorelix acetate (Cetrotide; Merck Serono, Darmstadt, Germany) was initiated once the leading follicle reached 13-14 mm in size. The final injection was administered on the day of the ovulation trigger. Oocyte maturation was induced by 250 µg of human chorionic gonadotropin (hCG) (Ovitrelle; Merck Serono, Germany) and 0.1 mg of triptorelin (Gonapeptyl; Ferring, Switzerland), and oocyte retrieval was scheduled 35 hours thereafter.

Laboratory Procedures and PGT-A

Metaphase II (MII) oocytes were fertilized by ICSI in G-MOPS™ fertilization medium (Vitrolife, Göteborg, Sweden) supplemented with 10% human serum albumin (HSA, Vitrolife). The embryos were cultured in one-step continuous single-culture media (Global® Total® LP, LifeGlobal™). The evaluation of embryo quality involved grading fresh cleavage-stage embryos produced by ICSI according to rigorous criteria[19]. Accordingly, embryos were categorized as top-quality (TQ) if they had reached the 7-10-cell stage on day 3 (64-66 hours) of *in vitro* culture, had exhibited symmetrical blastomeres free of multinucleation, and had demonstrated no more than 20% cytoplasmic fragmentation. Blastocyst morphology was assessed at two time points: days 5 (114 hours after ICSI) and 6 (138 hours after ICSI). This assessment encompassed an evaluation of three parameters, namely, the degree of blastocoel expansion, inner cell mass score, and TE score, utilizing the grading methodology established by Gardner and Schoolcraft[20]. Per standard protocol, biopsy and vitrification were not performed on blastocysts with CB or CC scores and a degree of expansion below 3. Assisted hatching via a laser pulse (RI Saturn 5, Cooper Surgical) was conducted on day 5 or 6 once the blastocyst reached a degree of expansion of 3 or higher. After re-expansion, TE biopsy was performed using the pulling method[21]. As a standard procedure, 3-5 cells were removed from the TE layer. The vitrification process for blastocysts adhered to standard protocols and used the Irvine Scientific Freeze Kit in conjunction with a custom-made straw carrier system.

PGT-A was performed using the Ion ReproSeq PGS Kit NGS platform (Thermo Fisher Scientific, USA) to screen for aneuploidy across all 24 chromosomes. The assay was performed using the Ion Chef and Ion S5 systems (Thermo Fisher Scientific, Inc., MA, USA), and the data were analyzed via Ion Reporter software (version 5.4). Embryos were systematically categorized as either euploid or aneuploid. Embryos exhibiting single-chromosome mosaicism, whether trisomy or monosomy, at frequencies below 50% were designated as euploid. This threshold is routinely accepted in our practice based on previously reported outcomes, which indicate that clinical pregnancy, implantation, and live-birth rates associated with ETs of blastocysts exhibiting low-level mosaicism (less than 50%) are comparable to those of euploid blastocysts[22]. Conversely, those with mosaic rates exceeding this threshold were classified as aneuploids. Furthermore, embryos characterized by two-chromosome mosaics or complex mosaic patterns were also classified as aneuploids.

Frozen Single-euploid Blastocyst Transfer and Luteal Phase Support

This study included only the first frozen ET cycle for each patient. In the context of endometrial preparation, a true natural-cycle follow-up approach was preferred for patients with regular menstrual cycles of 24 to 35 days over the preceding three months. In contrast, hormone replacement therapy (HRT) was the method of choice for individuals with irregular menstrual cycles or those unable to undergo serial ultrasound and serum hormone monitoring.

During true natural follow-up, serum estradiol and LH levels were monitored daily or every other day once the dominant follicle reached 16 mm. Ovulation was confirmed by a drop in serum estradiol together with a surge in LH. Subcutaneous progesterone (Prolutex; IBSA Institut Biochimique SA) was administered once daily between 4:00 pm and 7:00 pm on the same day as the estradiol decline, with the ET procedure scheduled for the sixth day after progesterone initiation.

The HRT protocol was performed as described elsewhere[23]. Progesterone support was continued through the eighth week of gestation for both transfer methods.

Outcome Measures

The primary outcome measures were organized into two main categories to enhance clarity and analytical hierarchy. Safety endpoints included the rates of premature LH rise (>10 mIU/mL) and cycle cancellations due to premature ovulation. We evaluated the euploidy rate per injected MII oocyte as the primary measure of effectiveness.

The secondary outcomes included the maturation and fertilization rates, the formation rate of day-3 TQ embryos, the blastulation rate, the euploid blastocyst rate per biopsy, and the proportion of cycles yielding a euploid blastocyst. Other secondary outcomes included rates of positive pregnancy tests, biochemical pregnancy losses, implantation, early miscarriages, and ongoing pregnancies.

Pregnancy was confirmed by a serum β-HCG concentration exceeding 5 IU/mL, measured 11 days after the frozen ET. Successful implantation was confirmed by the detection of a gestational sac on transvaginal ultrasound (TV-USG) at 6 weeks' gestation. An ongoing pregnancy was defined as the detection of a viable embryo with a fetal heartbeat at 12 weeks of gestation. Biochemical pregnancy loss is characterized by a temporary rise in hCG levels without the confirmation of a gestational sac via TV-USG. An early miscarriage is defined as a pregnancy loss that occurs before the 12th week of gestation after confirmation of successful implantation.

Statistical Analysis

The research methodology used frequency distributions to describe nominal and ordinal parameters, while scale parameters were characterized by means and standard deviations. To analyze differences in nominal and ordinal parameters, we employed the chi-square test, the likelihood ratio test, and Fisher's exact test. Additionally, the Kolmogorov-Smirnov test was conducted to assess the normality of the scale parameters. Since all parameter differences were non-normally distributed, the Mann-Whitney U test was used to evaluate differences among the scale parameters.

For the relational analysis, due to observed deviations from linearity, we applied Spearman's rho and the generalized linear model (logit) to investigate the association between the euploid blastocyst rate per injected MII oocyte and several pertinent variables[24]. All analyses were performed using the SPSS 25.0 platform for Windows, with a 95% confidence interval and a significance level of 0.05.

Results

The distribution of the entire cohort (n=548) based on age groups is as follows: 35-37 years, n=87 (16%); 38-40 years, n=156 (28%); 41-42 years, n=119 (22%); and >42 years, n=186 (34%). Regarding serum anti-Müllerian hormone (AMH) (ng/mL) levels, the distribution is as follows: 0.01-0.49, n=197 (36%); 0.50-1.19, n=188 (34%); and ≥1.20, n=163 (30%).

Table 1 presents the baseline characteristics and clinical parameters of the patient groups. Among these characteristics, mean female age, BMI, duration of infertility, number of previous failed attempts, type of infertility (primary or secondary), number of previous miscarriages, and AMH levels were comparable across groups (p>0.05). A premature LH rise was observed in 25 cycles (8.2%) within the fPPOS group and in 16 cycles (6.6%) in the GnRH antagonist group. No statistically significant difference was observed between the groups (p=0.302). Furthermore, the incidence of cycle cancellations due to premature ovulation was comparable between the groups, with 6 cancellations (2%) in the fPPOS group and 1 cancellation (0.4%) in the GnRH antagonist group (p=0.112).

Table 1. Baseline and clinical parameters of study groups

mean ± SD	fPPOS (n=307)	GnRH-ant (n=241)	p-value
Female age, years	40.91±2.92	40.54±2.82	0.092[a]
BMI (kg/m²)	23.57±4.29	23.39±4.16	0.676[a]
Duration of infertility, years	4.64±3.49	4.86±3.94	0.805[a]
No of previous failed attempts, n	2.49±1.81	2.35±1.94	0.289[a]
Type of infertility, n (%)			
Primary	116 (37.8)	98 (40.7)	0.275[b]
Secondary	191 (62.2)	143 (59.3)	
Previous miscarriages, n	1.07±1.34	0.86±1.16	0.057[a]
Previous live birth, n	0.16±0.43	0.23±0.50	*0.045[a]
AMH, ng/mL	1.09±1.07	1.08±1.30	0.590[a]
Antral follicle count, n	8.18±5.63	6.91±5.05	*0.001[a]
Sperm concentration, 10^6/mL	42.71±31.36	37.20±30.32	*0.026[a]
Duration of COH, days	10.51±1.83	9.83±1.97	*0.000[a]
Duration of LH suppression, days	5.57±1.29	4.59±1.12	*0.000[a]
Total gonadotropin dosage, IU	2669.17±901.97	2286.61±911.82	*0.000[a]
LH level on the trigger day, mIU/mL	5.96±4.95	4.65±4.17	*0.001[a]
Premature LH rise, >10 mIU/mL, n (%)	25 (8.2)	16 (6.6)	0.302[b]
Cancellation due to premature ovulation, n (%)	6 (2.0)	1 (0.4)	0.112[b]

[a]: Mann-Whitney U test, [b]: Fisher's exact test, GnRH: Gonadotropin-releasing hormone, fPPOS: Flexible progestin-primed ovarian stimulation, SD: Standard deviation, COH: Controlled ovarian hyperstimulation, BMI: Body mass index, LH: Luteinizing hormone, AMH: Anti-Müllerian hormone, *: A statistically significant difference was identified by p<0.05

In contrast, the fPPOS group demonstrated significantly higher antral follicle counts (AFC), sperm concentrations, and mean number of previous live births (p<0.05). This group also experienced significantly longer durations of COH (p<0.001) and LH suppression (p<0.001). Additionally, the fPPOS group required a higher total gonadotropin dosage (p<0.001) and exhibited elevated LH levels on the trigger day (p<0.005) compared with the GnRH antagonist group.

The embryological and genetic outcomes of the patient cohort are displayed in Table 2. The fPPOS group demonstrated significantly higher mean numbers of oocytes retrieved, MII oocytes, 2PN zygotes, and day 3-TQ embryos (p<0.05). However, the maturation rate, the fertilization rate, the blastulation rate, and other metrics such as the number of cycles resulting in zero blastocysts and zero euploid blastocysts, the number of cycles yielding at least one euploid blastocyst, the euploid blastocyst rate per biopsy, and the euploid blastocyst rate per injected MII were comparable between the groups (p>0.05).

The Spearman's rho correlation analysis, as presented in Table 3, indicated that the euploid blastocyst rate per injected MII oocyte was significantly correlated with female age, AMH levels, AFC, the number of oocytes retrieved, the number of MII oocytes, and the number of 2PN zygotes in both groups. BMI showed a negative correlation in the GnRH antagonist group (r=-0.166; p<0.05). Previous live birth, sperm concentration, durations of COH and LH suppression, and total gonadotropin dosage were not correlated with euploid blastocyst rate per injected MII oocyte in either group.

The Generalized Linear Model analysis demonstrated that both the maturation rate [odds ratio (OR)=0.114; p<0.05] and the blastulation rate (OR=0.194; p<0.01) were significant independent predictors of the euploid blastocyst rate per injected MII oocyte within the fPPOS group. Conversely, in the GnRH antagonist group, the blastulation rate (OR=0.132; p<0.01) was identified as the only significant independent variable influencing the outcome (Table 4).

Table 5 presents the outcomes associated with frozen ET cycles. The numbers of cycles in which at least one euploid blastocyst was obtained and frozen ET was performed in the fPPOS and GnRH antagonist groups were 98 (84.5%) and 65 (83.3%), respectively. The parameters compared, including the day embryos were transferred, endometrial thickness, and the rates of positive pregnancy tests, implantation, and ongoing pregnancy, did not reach statistical significance (p>0.05). In addition, the biochemical pregnancy loss rate and the early miscarriage rate were comparable (p=0.912).

Table 2. Embryological outcomes of study groups

mean ± SD	fPPOS (n=307)	GnRH-ant (n=241)	p-value
No. of oocytes retrieved, n	6.95±5.18	6.29±5.68	*0.025[a]
No. of MII, n	5.67±4.47	5.12±4.89	*0.026[a]
No. of 2PN, n	4.81±3.96	4.08±3.86	*0.005[a]
Maturation rate, %	0.81±0.21	0.80±0.24	0.917[a]
Fertilization rate, %	0.85±0.20	0.82±0.24	0.487[a]
No. of day 3 TQ embryos, n	2.85±2.78	2.18±2.25	*0.005[a]
Blastulation rate, %	0.47±0.31	0.53±0.35	0.059[a]
No. of cycles with zero blastocyst, n (%)	65 (21.17)	57 (23.65)	0.278[b]
No. of cycles with zero euploid blastocyst, n (%)	126 (52.07)	106 (57.61)	
No. of cycles with at least one euploid blastocyst, n (%)	116 (47.93)	78 (42.39)	0.291[c]
Embryo biopsy day			
D5	1.87±2.03	1.59±2.04	0.098[a]
D6	0.44±0.81	0.50±0.96	0.647[a]
Euploid blastocyst rate per biopsy, %	206/411 (50.12)	156/294 (53.06)	0.317[a]
Euploid blastocyst rate per injected MII, %	206/864 (23.84)	156/641 (23.34)	0.231[a]

[a]: Mann-Whitney U test, [b]: Fisher's exact test, [c]: Chi-square likelihood ratio, GnRH: Gonadotropin-releasing hormone, fPPOS: Flexible progestin-primed ovarian stimulation, SD: Standard deviation, MII: Metaphase II, TQ: Top-quality, *: A statistically significant difference was identified by p<0.05

Table 3. Spearman's rho correlation for effects of baseline and clinical parameters on euploid blastocyst rate per injected MII oocyte

Euploid blastocyst rate per injected MII oocyte	fPPOS		GnRH-ant	
	r	p	r	p
Women's age	-0.548**	0.000	-0.487**	0.000
BMI	-0.017	0.769	-0.166*	0.012
Previous live birth	-0.045	0.438	-0.052	0.428
AMH	0.161**	0.005	0.350**	0.000
Antral follicle count	0.231**	0.000	0.366**	0.000
Sperm concentration	0.041	0.481	0.047	0.478
Duration of COH	0.011	0.854	0.036	0.589
Duration of LH suppression	0.060	0.303	0.106	0.107
Total gonadotropin dosage	0.009	0.881	-0.030	0.646
No. of oocytes retrieved	0.233**	0.000	0.365**	0.000
No. of MII	0.264**	0.000	0.380**	0.000
No. of 2PN	0.289**	0.000	0.412**	0.000

*p<0.05, **p<0.01, COH: Controlled ovarian hyperstimulation, BMI: Body mass index, LH: Luteinizing hormone, GnRH: Gonadotropin-releasing hormone, fPPOS: Flexible progestin-primed ovarian stimulation, MII: Metaphase II, AMH: Anti-Müllerian hormone

Table 4. Generalized linear model (logit) for effects of clinical parameters on euploid blastocyst rate per injected MII for patient groups

Parameter	OR	Standard error	95% wald confidence interval		Hypothesis test		
			Lower	Upper	Wald X^2	df	p-value
fPPOS group							
(Intercept)	-0.345	0.2787	-0.891	0.201	1.534	1	0.216
Duration of COH	-0.007	0.0090	-0.024	0.011	0.534	1	0.465
Duration of LH suppression	0.001	0.0085	-0.016	0.017	0.004	1	0.947
Total gonadotropin dosage	0.033	0.0418	-0.049	0.115	0.605	1	0.437
LH level on the trigger day	-0.002	0.0023	-0.006	0.003	0.524	1	0.469
Maturation rate	0.114	0.0542	0.008	0.220	4.432	1	0.035*
Fertilization rate	0.095	0.0595	-0.022	0.212	2.546	1	0.111
No of day 3-TQ embryos	-0.007	0.0067	-0.020	0.006	1.111	1	0.292
Blastulation rate	0.194	0.0382	0.119	0.269	25.814	1	0.000*
Embryo biopsy day 5	0.016	0.0097	-0.003	0.035	2.782	1	0.095
Embryo biopsy day 6	0.001	0.0139	-0.027	0.028	0.001	1	0.971
(Scale)	0.025	0.0021	0.022	0.030			
GnRH-Ant group							
(Intercept)	0.460	0.2987	-0.126	1.045	2.367	1	0.124
Duration of COH	0.001	0.0110	-0.021	0.022	0.002	1	0.964
Duration of LH suppression	0.010	0.0140	-0.017	0.038	0.541	1	0.462
Total gonadotropin dosage	-0.061	0.0460	-0.151	0.029	1.770	1	0.183
LH level on the trigger day	0.002	0.0034	-0.005	0.008	0.219	1	0.640
Maturation rate	-0.103	0.0597	-0.220	0.014	2.990	1	0.084
Fertilization rate	0.014	0.0571	-0.098	0.126	0.063	1	0.802
No of day 3-TQ embryos	0.014	0.0088	-0.003	0.031	2.616	1	0.106
Blastulation rate	0.132	0.0369	0.060	0.205	12.862	1	0.000*
Embryo biopsy day 5	0.015	0.0097	-0.004	0.034	2.421	1	0.120
Embryo biopsy day 6	-0.003	0.0143	-0.030	0.025	0.031	1	0.860
(Scale)	0.027	0.0026	0.023	0.033			

*: A statistically significant difference was identified by p<0.05, COH: Controlled ovarian hyperstimulation, LH: Luteinizing hormone, TQ: Top-quality, GnRH: Gonadotropin-releasing hormone, fPPOS: Flexible progestin-primed ovarian stimulation, OR: Odds ratio, MII: Metaphase

Table 5. Frozen embryo transfer outcomes

Parameter	fPPOS	GnRH-ant	p-value
Day of embryos transferred			
D5	85 (27.7)	55 (22.8)	0.422[a]
D6	13 (4.2)	10 (4.1)	
Endometrial thickness, mm	9.75±6.81	9.35±1.84	0.483[b]
Positive pregnancy test rate, n (%) (per transfer)	78/101 (77.23)	49/65 (75.38)	0.314[a]
Implantation rate, n (%) (per transfer)	67/101 (66.34)	45/65 (69.23)	0.415[c]
Ongoing pregnancy rate, n (%) (per transfer)	55/101 (54.45)	35/65 (53.84)	0.538[d]
Pregnancy loss rate, % (per transfer)			
Biochemical pregnancy loss, n (%)	11/101 (10.89)	5/65 (7.69)	0.912[c]
Early miscarriage, n (%)	12/67 (17.91)	9/45 (20.00)	

[a]: Pearson's chi-square test, [b]: Mann-Whitney U test, [c]: Chi-square likelihood ratio, [d]: Fisher's exact test, SD: Standard deviation, *: A statistically significant difference was identified by p<0.05, GnRH: Gonadotropin-releasing hormone, fPPOS: Flexible progestin-primed ovarian stimulation

Discussion

This research is the first comprehensive study to present detailed data on embryological processes and embryo ploidy in the fPPOS group. We analyzed the outcomes associated with the fPPOS and GnRH antagonist protocols, focusing on clinical, embryological, and PGT-A results. The number of cycles in which a premature LH rise occurred and the number of cycles cancelled due to premature ovulation were similar across both groups. Although the fPPOS group produced greater numbers of retrieved oocytes, 2PN zygotes, and day 3-TQ embryos, we did not find significant differences in maturation, fertilization, and blastulation rates, or in euploid blastocyst rates per biopsy and per injected MII oocyte.

Univariate correlation analysis indicated that several demographic, embryological, and clinical parameters showed similar associations with the euploid blastocyst rate per injected MII oocyte in both groups, except for increased BMI, which showed a negative correlation in the GnRH antagonist group. The multivariate regression analysis revealed that factors such as the duration of COH and LH suppression, total gonadotropin dosage, LH levels on the trigger day, the number of day 3-TQ embryos, and the embryo biopsy day (D5 or D6) did not significantly influence the euploid blastocyst rate per injected MII oocyte in either group. Additionally, the pregnancy outcomes were comparable between the two groups.

The flexible approach was first introduced for hyperresponder donor patients and compared with the GnRH antagonist protocol[25]. A similar comparison was conducted in a distinct population of patients with diminished ovarian reserve who underwent oocyte freezing[2,26]. In both the previously mentioned studies and a recently published randomized non-inferiority trial[27], no significant differences were observed between the fPPOS and GnRH antagonist groups regarding total gonadotropin dosage requirements and early embryological parameters. Despite the limited use of MPA in our study, the fPPOS group demonstrated a prolonged duration of COH and a higher total gonadotropin dose requirement, which contrasts with the existing literature. The intentional delay of at least one day in triggering maturation for the fPPOS group, aimed at optimizing the number of retrieved oocytes, may have contributed to these outcomes. Furthermore, we observed significantly higher numbers of retrieved oocytes, MII oocytes, 2PN zygotes, and D3-TQ embryos in the fPPOS group. However, the maturation, fertilization, and blastulation rates were comparable between the groups, which is consistent with the literature[17]. This finding can primarily be attributed to the higher baseline AFC in the fPPOS group compared to the GnRH antagonist group (8.18±5.63 vs. 6.91±5.05; p<0.01). Additionally, the mild pituitary suppression theory proposed by Vidal et al.[28] may have contributed to this outcome. This theory was validated in this study, and the observed significance was attributable to higher serum LH levels measured on the maturation trigger day in the fPPOS group than in the GnRH antagonist group (5.96±4.95 versus 4.65±4.17; p<0.01). A similar phenomenon was documented in an RCT conducted by Chen et al.[3], which employed the conventional PPOS protocol (3.17±4.84 versus 2.59±1.77; p=0.023). Moreover, a recently published RCT by Cai et al.[27] utilizing the fPPOS protocol corroborated these findings [2.9 (1.9-4.4) versus 2.5 (1.4-3.7); p=0.017].

The euploidy rate is a robust indicator of embryo quality. The transfer of euploid embryos notably enhances live birth rates per transfer, particularly in cases involving women aged over 35 years[29]. The identification of similar or elevated euploidy rates in the fPPOS group compared with those in the GnRH antagonist or agonist groups would significantly substantiate the treatment's reliability. In a recent review, the topic was assessed under the subtitle "Does PPOS Affect Developmental Potential of Oocytes"[17]. The collective findings of one prospective[30] and three retrospective studies[31-33] suggest that the euploidy rate per biopsy was similar in both the conventional PPOS and GnRH antagonist groups. However, one study in which the euploidy rate was claimed to be lower in the PPOS group (5.4% versus 26.7%; p=0.006) was criticized because of its small sample size[34]. Conversely, a current retrospective cohort study reported significantly high rates of both euploid blastocysts per biopsy (54.94% versus 40.88%; p=0.015) and euploid blastocysts per MII oocyte (15.48% versus 10.47%; p=0.013) in the conventional PPOS group compared to GnRH antagonist group; however, these findings may also be questioned due to the small sample size involved[35]. Apart from these, a recent prospective study reported a comparable mean number of biopsied blastocysts, a mean number of euploid blastocysts, and a euploidy rate per biopsied blastocyst across treatment protocols[28]. Consistent with these findings, our study did not identify any significant differences between treatment groups in the euploid blastocyst rate per biopsy (50.1% vs. 53.1%; p>0.05) or per injected MII oocyte (23.8% vs. 23.3%; p>0.05).

The pregnancy outcomes noted in this study are consistent with those found in a previously published review[17] and a meta-analysis[36]. Moreover, recent findings from a retrospective study[35] and an RCT[27] lend further support to these conclusions. The fPPOS protocol yielded results comparable to the widely used GnRH antagonist protocol in terms of implantation rates (66.34% vs. 69.23%; p=0.415) and ongoing pregnancy rates (54.45% vs. 53.84%; p=0.538) per transfer. In light of these findings, we can conclude that ETs utilizing euploid blastocysts obtained through the fPPOS protocol are not inferior to those achieved with the GnRH antagonist protocol.

This study demonstrates its strength through a targeted focus on a patient group that faces significant challenges in treatment management and often has suboptimal outcomes.

Additionally, it provides a thorough, first-time evaluation of the fPPOS protocol, particularly with respect to embryological and PGT-A outcomes.

Study Limitations

This study provides a snapshot of daily practice. Although maturation, fertilization, blastulation, and euploidy rates per biopsy and per injected MII oocyte were similar across groups according to a comprehensive multivariate analysis, the higher AFC, prolonged duration of COH, and higher total gonadotropin dose in the fPPOS group may still influence embryological yield. Another limitation of this study is its retrospective design, which necessitates a cautious interpretation of the findings. To substantiate these results, future large-scale randomized trials with adequate sample sizes will be essential. Furthermore, research calculating cumulative live birth rates using robust statistical methods will yield more valuable insights into comparing pregnancy outcomes between the two groups.

Conclusion

The fPPOS protocol, utilizing MPA, offers a straightforward, convenient, and effective treatment that requires fewer pills and is more patient-friendly. Flexible application does not adversely affect embryological outcomes or PGT-A results, nor does it increase the risk of cycle cancellations due to premature ovulation. Furthermore, no negative effects on pregnancy outcomes were observed. Therefore, fPPOS may be recommended as the preferred option in cases where fresh ET does not immediately follow ovarian stimulation.

Ethics

Ethics Committee Approval: The Institutional Review Board of Acıbadem Hospital (date: 02.01.2025) approved the research.

Informed Consent: In this study, informed consent was not considered necessary because the data used were entirely anonymized.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.E.T., Concept: N.E.T., Design: N.E.T., E.B., Data Collection or Processing: N.E.T., H.B., Analysis or Interpretation: E.K., Literature Search: N.E.T., S.Ö., Writing: N.E.T.

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