Obstetrics & Gynecology: Open Access
Nakagawa K et al. Gynecol Obstet Open Acc 9: 267.
www.doi.org/10.29011/2577-2236.100267
www.gavinpublishers.com

Research Article

Higher Late-Follicular Progesterone Per Follicle is Associated with A Lower Euploid Blastocyst Rate but Comparable Pregnancy Outcomes After Single Euploid Frozen Blastocyst Transfer

Koji Nakagawa*, Takashi Horikawa, Keisuke Shiobara, Shota Hatakeyama, Hideaki Watanabe, Satoru Takamizawa, Rikikazu Sugiyama

Center for Reproductive Medicine and Implantation Research, Sugiyama Clinic Shinjuku, Tokyo, Japan.

***Corresponding author:** Koji Nakagawa, Center for Reproductive Medicine and Implantation Research, Sugiyama Clinic Shinjuku, 1-19-6, Nishi-Shinjuku, Shinjuku-ku, Tokyo, 160-0023, Japan.

Citation: Nakagawa K, Horikawa T, Shiobara K, Hatakeyama S, Watanabe H, et al. (2026). Higher Late-Follicular Progesterone Per Follicle is Associated with A Lower Euploid Blastocyst Rate but Comparable Pregnancy Outcomes After Single Euploid Frozen Blastocyst Transfer. Gynecol Obstet Open Acc 9: 267. DOI: https://doi.org/10.29011/2577-2236.100267.

Received Date: 29 April 2026; **Accepted Date:** 11 May 2026; **Published Date:** 16 May 2026

Abstract

Objective: To determine whether late–follicular phase progesterone elevation during ovarian stimulation reduces pregnancy outcomes by lowering euploid embryo yield and/or impairing the implantation potential of euploid blastocysts. **Methods:** This retrospective observational study included 2,559 PGT-A cycles (January 2021–December 2025) limited to ICSI. Progesterone per follicle (P/fol) was calculated as serum progesterone on the trigger day divided by the number of follicles ≥18 mm. A receiver operating characteristic curve was used to identify a P/fol cut-off for obtaining ≥1 euploid blastocyst. Euploid rates and pregnancy outcomes after single euploid frozen–thawed blastocyst transfer were compared between P/fol groups; mosaic embryo transfers were excluded. Multivariable logistic regression adjusted for relevant confounders. **Results:** The optimal cutoff was P/fol = 0.136 (AUC 0.626). Euploid rate was significantly lower in the P/fol-high group than in the P/fol-low group (17.4% vs 22.3%), remaining significant after adjustment. Among transfers of euploid blastocysts (542 vs 617 cycles), hCG-positive, clinical pregnancy, and ongoing pregnancy rates did not differ between groups. **Conclusion:** Elevated late-follicular P/fol was associated with a lower euploid blastocyst rate in PGT-A cycles. However, among cycles in which a euploid blastocyst was transferred, pregnancy outcomes were not significantly different according to the P/fol category. These findings suggest that elevated P/fol may primarily affect embryo yield rather than the reproductive potential of euploid blastocysts, although external validation is required.

Keywords: Euploidy; Frozen embryo transfer; Ovarian stimulation; PGT-A; Progesterone

Introduction

In the 1990s, the standard ovarian stimulation protocol for in vitro fertilization and embryo transfer was the gonadotropin-releasing hormone (GnRH) agonist long protocol. Because this approach suppresses pituitary gonadotropin secretion using a GnRH agonist, premature ovulation before oocyte retrieval became very rare, and it greatly contributed to the widespread use of assisted reproductive technology (ART) in the 1990s. At that time, fresh embryo transfer was the main strategy. However, it was well known that when an increase in serum progesterone was observed during ovarian stimulation, implantation rates after fresh embryo transfer were

significantly reduced [1-3]. This reduction was thought to be caused by progesterone-induced decidualization of endometrial stromal cells, leading to a mismatch between the endometrial window of implantation and the embryos created after oocyte retrieval [4]. Therefore, when serum progesterone rose during stimulation, it was considered that freezing all embryos and performing transfer in a subsequent cycle—timed to match embryo stage with the number of days after ovulation—could improve implantation rates [1]. However, more recently, it has been reported that an elevation in serum progesterone during the late follicular phase of ovarian stimulation is associated with a significant reduction not only in pregnancy rates after frozen–thawed embryo transfer but also in cumulative live birth rates [5].

Furthermore, we recently performed a similar analysis. We calculated the serum progesterone level per follicle on the day of final oocyte maturation trigger (P/fol) and found that pregnancy rates after frozen–thawed embryo transfer were significantly lower when blastocysts derived from cycles with elevated P/fol were used, even after accounting for factors that may affect pregnancy outcomes (female age, AMH, previous pregnancy, and the number of follicles ≥18 mm) [6]. Because both our study and the report by Rocca et al [5]. evaluated frozen–thawed embryo transfer cycles, a mismatch between embryo developmental stage and endometrial receptivity cannot plausibly explain the reduced pregnancy rates; thus, the underlying mechanism has remained unclear.

Therefore, to investigate the mechanism by which an elevation in progesterone during the late follicular phase reduces pregnancy rates, we calculated the progesterone level per developing follicle using late-follicular progesterone measurements obtained during ovarian stimulation (P/fol). We then evaluated the association between P/fol and the proportion of euploid embryos and further assessed implantation rates of those euploid embryos.

Materials and Methods

Study population and cycle selection

This was a retrospective observational study. We evaluated cycles in which oocyte retrieval for PGT-A was performed between January 2021 and December 2025, using data from hormone levels and the number of developing follicles during ovarian stimulation, post-fertilization culture outcomes, and pregnancy outcomes after transfer of euploid blastocysts derived from these cycles. The retrospective use of patients' clinical data for research and publication in a non-identifiable manner was approved in advance by the institutional ethics committee (18-0001).

Fertilization was limited to intracytoplasmic sperm injection (ICSI). Fresh ejaculated sperm or frozen–thawed sperm was used. Cycles requiring surgical sperm retrieval (MESA or TESE) and cycles requiring oocyte activation due to fertilization failure were excluded.

During the study period, ovarian stimulation was initiated and oocyte retrieval for PGT-A was performed in 3,958 cycles. Because this study focused on ICSI cycles for PGT-A and because the number of MII oocytes can be assessed more reliably in ICSI cycles, 959 conventional IVF and split cycles were excluded. We further excluded 151 cycles in which no embryos were obtained or no blastocysts suitable for biopsy were available, and 289 cycles with missing data. The final study population comprised 2,559 cycles.

Ovarian Stimulation

Baseline assessment was performed on menstrual cycle day (MC) 2–3 and included serum estradiol (E2), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) measurements and transvaginal ultrasonography. Stimulation protocols were categorized as natural, minimal, mild, GnRH antagonist, and modified progestin protocol. Less frequently used GnRH agonist long and short protocols were grouped as "other."

- Minimal: clomiphene citrate (CC; Clomid®, Fuji Pharma, Tokyo, Japan) 50–100 mg/day from MC3 to the day before trigger.
- Mild: CC 100 mg/day or letrozole (LTZ; letrozole®, Fuji Pharma, Tokyo, Japan) 5 mg/day for 7 days (MC3–MC9), plus recombinant FSH (rFSH; Gonal-F®, Merck Biopharma, Tokyo, Japan; Rekovelle®, Ferring Pharmaceuticals, Tokyo, Japan) with dose adjustments based on MC3 FSH level, antral follicle count, and AMH level [7].
- Progestin protocol: as in the mild protocol, plus oral medroxyprogesterone acetate (Hysron®, Kyowa, Tokyo, Japan) 10 mg twice daily from MC6 until trigger [8].
- GnRH antagonist: CC 100 mg/day or LTZ 5 mg/day for 7 days, with daily rFSH 150–300 IU starting on MC3; ganirelix acetate (Ganirest®, Organon, Tokyo, Japan) 0.25 mg/day was added according to monitoring and continued until trigger.

Final oocyte maturation was triggered when ≥2 follicles reached ≥18 mm using one of the following: recombinant hCG (Ovidrel®, Merck Biopharma, Tokyo, Japan) 250 μg [9], intranasal GnRH agonist (Busererine®, Fuji Pharma, Tokyo, Japan) 300 μg administered twice 30 minutes apart, or a dual trigger.

The distribution of stimulation protocols is shown in Figure 1. Overall, 57.6% of cycles used the progestin protocol, followed by mild stimulation (33.8%), the GnRH antagonist protocol (6.8%), minimal stimulation (1.4%), and other protocols (0.4%).

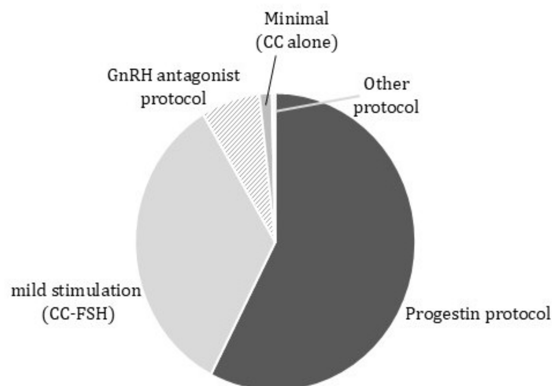

Figure 1: The distribution of ovarian stimulation protocols

The distribution of ovarian stimulation protocols is shown. Overall, 57.6% of cycles used the progestin protocol, followed by mild stimulation (CC–FSH; 33.8%), the GnRH antagonist protocol (6.8%), minimal stimulation (CC alone; 1.4%), and other protocols (0.4%).

Hormone assays

Baseline serum E2, FSH, and LH levels on MC3 were measured using commercially available chemiluminescent enzyme immunoassay (CLEIA) kits (AIA-Pack CL®, Tosoh Corporation, Tokyo, Japan). Serum E2, LH, and progesterone (P) levels were measured using the same analytical platform on the day of final oocyte maturation trigger using the same CLEIS platform. Serum anti-Müllerian hormone (AMH) was measured using a commercially available immunoassay kit (VIDAS® AMH Assay, bioMérieux Japan, Tokyo, Japan).

Oocyte pickup and embryo culture

Oocyte pickup (OPU) was performed transvaginally under ultrasound guidance using 20- or 21-gauge needles, with follicular aspiration by either manual syringe or pump. Anesthesia (intravenous sedation, local anesthesia, or no anesthesia with NSAID analgesia) was selected according to patient preference. Cumulus–oocyte complexes were denuded immediately after retrieval, and nuclear maturation was assessed. Semen was obtained as either fresh ejaculate or previously cryopreserved ejaculate. To minimize heterogeneity, the present analysis was restricted to ICSI cycles, and fertilization was performed using Piezo-ICSI [10]. Embryos were cultured individually to the blastocyst stage under reduced-oxygen conditions (5% O_2, 6% CO_2, and 89% N_2) in a time-lapse incubator [11].

TE Biopsy, Next-generation assay, and Blastocysts cryopreservation

All patients were scheduled to undergo PGT-A because of a history of recurrent pregnancy loss (RPL), repeated implantation failure (RIF), and/or advanced maternal age (≥40 years).

Trophectoderm (TE) biopsy was performed on blastocysts with a quality grade of ≥4BB according to Gardner's classification system [12]. In addition, blastocysts with TE grade "A" or "B" were considered eligible for biopsy even when the inner cell mass (ICM) grade was "C". The TE biopsy and next-generation sequencing (NGS) procedures used in our laboratory have been published previously [13] and are briefly summarized here. TE biopsy was performed using a mechanical blunt-dissection technique, without laser-assisted TE cutting; the laser was used only to open the zona pellucida. Using time-lapse monitoring, expanded blastocysts with an adequate number of TE cells were selected. First, a small opening was created in the zona pellucida using an infrared diode laser (Saturn 5™ Active, Cooper Surgical, CT, USA), followed by TE breaching to induce blastocele collapse. A biopsy pipette (Kitazato, Shizuoka, Japan) was then introduced through the opening into the perivitelline space, and TE cells were aspirated. Approximately 5–10 TE cells were isolated by mechanical blunt dissection [14]. All procedures were performed using micromanipulation instruments with embryos maintained in droplets of PGD biopsy medium (Global, LifeGlobal, USA).

The isolated TE cells were washed twice in sterile phosphate-buffered saline (PBS) supplemented with 1% polyvinylpyrrolidone (PVP), transferred into a 0.2-mL PCR tube containing 2.5 µL PBS, and stored at −20°C until DNA analysis. Biopsy samples were sent either to Kitazato Biolab (Tokyo, Japan) for whole-genome amplification (WGA) using the SurePlex DNA Amplification System (Illumina, CA, USA) or to the Igenomix laboratory (Tokyo, Japan) for equivalent processing. Amplified DNA was analyzed by NGS using either the MiSeq system (Illumina, CA, USA) or EmbryoMap (Vitrolife, Sweden). Copy number variation (CNV) profiles were generated using BlueFuse Multi software (Illumina, CA, USA).

After biopsy, all remaining blastocysts were cryopreserved by vitrification using Kitazato Vitrification Medium VT505 and Cryotop® (Kitazato, Tokyo, Japan) until PGT-A results were available and ploidy status (euploid, mosaic, or aneuploid) was determined.

Frozen–thawed embryo transfer and Clinical outcomes

Blastocysts were thawed according to standard protocols. Endometrial preparation was performed using either ovulatory cycles (natural or letrozole-induced) or hormone-replacement cycles [15]. A single euploid blastocyst was transferred into the uterine cavity under transvaginal ultrasound guidance using a soft

embryo transfer catheter (Kitazato ET catheter; Kitazato Supply, Shizuoka, Japan). For luteal phase support, vaginal progesterone (90 mg of Crinone gel®,Merck Biopharma, Tokyo or 200 mg/day of Lutinas®, Ferring Pharmaceuticals, Tokyo, Japan) and/or oral dydrogesterone (Duphaston® 20–30 mg/day) was administered from 3 days before embryo transfer until the pregnancy test.

Serum hCG was measured 9–10 days after transfer; an hCG level >10 IU/L was considered positive. Clinical pregnancy was defined as the presence of an intrauterine gestational sac on ultrasound 16–17 days after transfer. Ongoing pregnancy was defined as a pregnancy continuing beyond 12 weeks of gestation. To minimize embryo-related confounding, cycles involving transfer of mosaic embryos were excluded from the present analysis.

Statistical analysis

Clinical data were analyzed using Fisher's exact test for categorical variables and nonparametric tests for continuous variables, as appropriate. To determine the P/fol threshold for obtaining at least one blastocyst, we constructed a receiver operating characteristic (ROC) curve and identified the optimal cutoff value using EZR.

Multivariable logistic regression models were constructed to identify independent factors associated with pregnancy outcomes after frozen–thawed embryo transfer (FET) using euploid blastocysts, while adjusting for potential confounders. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A two-sided p value <0.05 was considered statistically significant.

The primary outcome was the embryo-level euploid status among TE-biopsied blastocysts. Because multiple blastocysts could be derived from the same stimulation cycle, logistic regression models were fitted using generalized estimating equations or mixed-effects logistic regression to account for clustering within cycles. As a secondary cycle-level outcome, we evaluated the probability of obtaining at least one euploid blastocyst per retrieval cycle.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of R Commander that includes statistical functions frequently used in biostatistics [16].

Results

The distribution of ovarian stimulation protocols in the 2,559 cycles is shown in Figure 1. The progestin protocol was the most frequently used (57.6%, 1,473/2,559), followed by the CC–FSH protocol (33.8%, 964/2,559). The GnRH antagonist protocol accounted for 6.8% (174/2,559), clomiphene alone for 1.4% (36/2,559), and other protocols (natural cycle and short protocol) for 0.4% (10/2,559).

Baseline characteristics of the overall study population are summarized in Table 1. Mean (±SD) female age was 40.4 ± 4.1 years, male age was 42.4 ± 7.7 years, and BMI was 21.1 ± 2.9 kg/m². The number of prior oocyte retrieval was 4.9 ± 5.3 and the number of previous embryo transfer was 1.3 ± 2.0; 64.3% of patients were nulliparous (1,645/2,559). On the day of trigger, the number of follicles ≥18 mm was 6.2 ± 4.3 and the number of oocytes retrieved was 7.7 ± 6.1. Serum E2 and progesterone (P) levels on the trigger day were 1,130.0 ± 1,027.5 and 0.97 ± 0.84, respectively. When the trigger-day P level was divided by the number of follicles ≥18 mm, the progesterone level per follicle (P/fol) was 0.20 ± 0.18. A total of 8,226 blastocysts underwent trophectoderm biopsy; the mean number of biopsied blastocysts per cycle was 3.2 ± 2.9. The total number of euploid blastocysts was 1,644, yielding an overall euploid rate of 20.0% (1,644/8,226) (Table 2).

Table 1: Characteristics of all ICSI participants

Cycles, n	2,559
Female age, years*	40.4 ± 4.1
Male age, years*	42.4 ± 7.7
BMI, kg/m^2 *	21.1 ±2.9
AMH, ng/mL*	2.1± 2.1
Prior OPU[#1], n*	4.9 ± 5.3
Previous embryo transfer, n*	1.3 ± 2.0
No previous delivery, n (%)	1,645 (64.3)
Follicle >18mm on trigger,	6.2 ± 4.3
Retrieved oocytes, n*	7.7 ± 6.1
Estradiol level on trigger, ng/ml*	1133.0 ± 1027.5
Progesterone level on trigger, ng/ml*	0.97 ± 0.84
P4/fol[#2], ng/mL per follicle*	0.20 ±0.18

*mean ± SD
[#1]OPU: oocyte pick-up, [#2] P/fol: progesterone level per a follicle

Table 2: PGT-A outcomes

Number of trophectoderm (TE) biopsied blastocysts, n	8,226
Mean number of TE-biopsied blastocysts, n*	3.2±2.9
Number of euploid blastocysts, n	1,644
Mean number of euploid blastocysts, n*	0.6 ± 1.3
Euploid rate, %	20.0

Abbreviations: TE, trophectoderm
*Values are presented as mean ± SD unless otherwise indicated.

Next, to derive a P/fol cutoff for the cycle-level outcome of obtaining at least one euploid blastocyst, we constructed an ROC curve and identified the optimal threshold using the Youden index.

Citation: Nakagawa K, Horikawa T, Shiobara K, Hatakeyama S, Watanabe H, et al. (2026). Higher Late-Follicular Progesterone Per Follicle is Associated with A Lower Euploid Blastocyst Rate but Comparable Pregnancy Outcomes After Single Euploid Frozen Blastocyst Transfer. Gynecol Obstet Open Acc 9: 267. DOI: https://doi.org/10.29011/2577-2236.100267.

The discriminatory ability of P/fol for predicting the cycle-level acquisition of at least one euploid blastocyst was modest, as reflected by an AUC of 0.626 (95% CI, 0.603–0.646; sensitivity 0.542; specificity 0.635) (Figure 2). Therefore, the cutoff of 0.136 should be interpreted as an exploratory threshold rather than a clinically definitive cutoff. Using this cutoff, cycles were categorized into a P/fol-low group (P/fol <0.136) and a P/fol-high group (P/fol ≥0.136), and ART outcomes and PGT-A results were compared between groups.

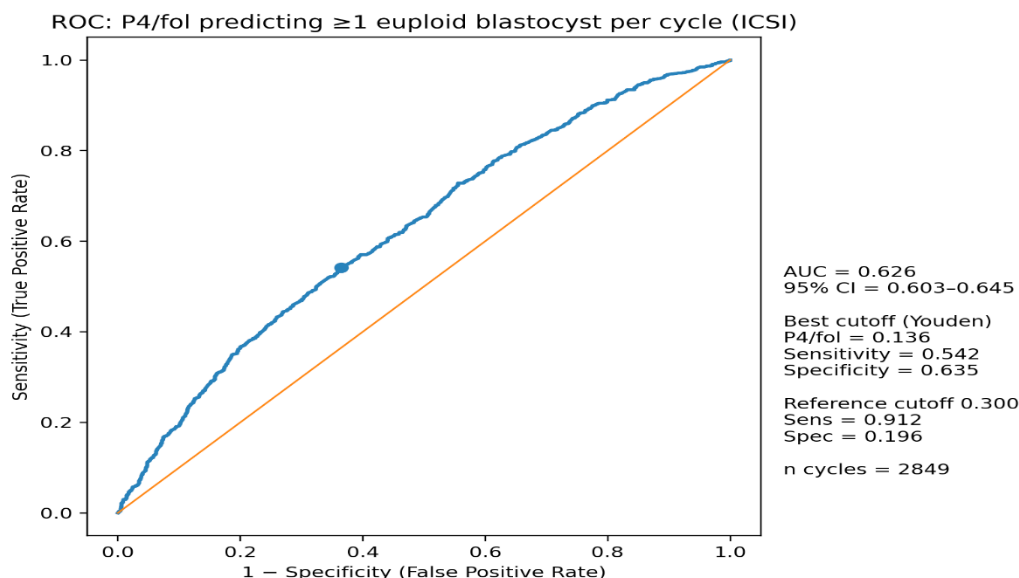

Figure 2: Receiver operating characteristic (ROC) curve of P4/fol for predicting the presence of at least one euploid blastocyst per cycle (ICSI cycles).

The ROC curve evaluates the ability of progesterone per follicle on the trigger day (P4/fol) to predict obtaining ≥1 euploid blastocyst per cycle. The optimal cutoff determined by the Youden index was P4/fol = 0.136 (AUC = 0.626; 95% CI, 0.603–0.645), with a sensitivity of 0.542 and specificity of 0.635. For reference, a cutoff of 0.300 yielded a sensitivity of 0.912 and specificity of 0.196. *n* = 2,559 cycles.

Baseline characteristics of the two groups are shown in Table 3. There were 1,068 cycles in the P/fol-low group and 1,491 cycles in the P/fol-high group. Female and male ages were significantly lower in the P/fol-low group. BMI, AMH, and trigger-day E2 were significantly higher in the P/fol-low group, whereas trigger-day P was significantly lower. The number of follicles ≥18 mm on the trigger day, the number of oocytes retrieved, the number of blastocysts obtained, and the number of good-quality blastocysts were all significantly greater in the P/fol-low group. The euploid rate was 22.3% (956/4,278) in the P/fol-low group and 17.4% (688/3,948) in the P/fol-high group; the P/fol-low group had a significantly higher euploid rate in both univariable and multivariable analyses (adjusting for female age, AMH, BMI, male age, trigger-day E2, and number of oocytes retrieved) (univariable: OR [95% CI], 0.493 [0.412–0.589], p<0.001; multivariable: aOR [95% CI], 0.796 [0.648–0.977], p=0.029, Table 4).

Pregnancy outcomes after transfer of euploid blastocysts derived from these stimulation cycles are shown in Table 5. A total of 542 transfers were performed in the P/fol-low group and 617 in the P/fol-high group. The hCG-positive rate was 73.2% in the P/fol-low group and 68.2% in the P/fol-high group, with no significant difference between groups (OR, 0.785; 95% CI, 0.609–1.013; p=0.070). Similarly, clinical pregnancy rates did not differ significantly (64.2% vs. 59.8%; OR, 0.829; 95% CI, 0.654–1.053; p=0.130). Ongoing pregnancy rates were 57.0% in the P/fol-low group and 54.0% in the P/fol-high group, again without a significant between-group difference (OR, 0.884; 95% CI, 0.701–1.115; p=0.314).

Table 3: Comparison of ART outcomes between the high and low P/fol groups

	P/fol-low group (P/fol<0.136)	P/fol-high group (P/fol ≥0.136)	P
Cycle, n	1,068	1,491	-
Female age, years	40.5 ± 4.3	41.0 ±3.8	<0.001
Male age, years	41.8 ± 8.0	42.9 ±7.4	<0.001
BMI, kg/m^2	21.2 ± 2.9	20.9 ± 2.9	0.011
AMH, ng/mL	2.7 ± 2.2	1.7 ±1.8	<0.001
Estradiol level on trigger, pg/ml	1181.5 ± 1138.5	1098.4 ± 938.3	<0.001
Progesterone level on trigger, ng/ml	0.71 ± 0.40	1.15 ± 1.01	<0.001
Follicle on trigger, n	8.0 ± 4.3	4.8 ±3.8	<0.001
Retrieved oocytes, n	9.3 ± 6.0	6.6 ±5.9	<0.001
P4/fol[#1], ng/mL per follicle	0.09 ± 0.03	0.46 ± 1.01	<0.001
Blastocysts, n	5.5 ± 4.7	3.7 ± 3.7	<0.001
Good-quality blastocyst, n	2.3 ±2.5	1.4 ±1.9	<0.001
Biopsied blastocyst, n	4,278	3,948	-
TE biopsied, n	4.0 ± 3.2	2.7 ± 2.5	<0.001
Euploid embryo	956	688	-
Euploid rate, %	22.3	17.4	<0.001

Values are mean ± SD. P-values are for descriptive purposes (Welch's t-test).
adjusting for female age, AMH, BMI, male age, trigger-day E2, and number of oocytes retrieved

Table 4: Logistic regression analyses of the association between high P/fol and euploid blastocyst formation.

Outcome	Variable	Univariable OR	95% CI	P value	Multivariable aOR	95% CI	P value
Euploid blastocyst formation	High P/fol group	0.493	0.412-0.589	<0.001	0.796	0.648-03977	0.029

The low P/fol group was used as the reference group. The multivariable model was adjusted for clinically relevant confounders, including female age, AMH level, prior pregnancy history, and the number of follicles ≥18 mm, as appropriate.

Table 5: Pregnancy outcomes after single euploid frozen-thawed blastocyst transfer according to P/fol category.

	P/fol Low group	P/fol High group	Odds ratio	95%CI	P
Cycle, n	542	617	-	-	-
hCG-positive, n	397	421	-	-	-
hCG-positive rate, %	73.2	68.2	0.785	0.609-1.013	0.070
Clinical pregnancy, n	348	369	-	-	-
Clinical pregnancy rate, %	64.2	59.8	0.829	0.654-1.053	0.130
Ongoing pregnancy, n	309	333	-	-	-
Ongoing pregnancy rate, %	57.0	54.0	0.884	0.701-1.115	0.314

Discussion

We recently reported that an elevated progesterone level per developing follicle during the late follicular phase of ovarian stimulation is an independent risk factor for reduced pregnancy rates, even when blastocysts from that cycle are cryopreserved and transferred in a subsequent frozen–thawed embryo transfer cycle [6]. To explore the underlying reason, we examined the proportion of euploid embryos among blastocysts generated from oocytes retrieved from follicles that developed under these conditions.

First, we sought to determine a P/fol cutoff value for obtaining at least one euploid embryo. Because higher P/fol was expected to be inversely associated with euploid blastocyst acquisition, the ROC analysis was performed to evaluate the ability of lower P/fol values to predict the presence of at least one euploid blastocyst per cycle. Using ROC curve analysis, the optimal threshold was P/fol = 0.136 (AUC = 0.626; 95% CI, 0.603–0.646; sensitivity, 0.542; specificity, 0.635). Therefore, we divided cycles into two groups using this cutoff: the P/fol-high group (P/fol ≥0.136) and the P/fol-low group (P/fol <0.136), and compared euploid rates between groups. Notably, this cutoff was derived in an exploratory manner based on ROC analysis and will require internal and/or external validation in future studies.

Our findings showed that the euploid rate was significantly lower in the P/fol-high group than in the P/fol-low group (univariable analysis: OR [95% CI], 0.493 [0.412–0.589], p<0.001). This association remained significant after adjustment, indicating that elevated P/fol is an independent risk factor for a reduced euploid rate, irrespective of female age, AMH, and other covariates (multivariable analysis: adjusted OR [95% CI], 0.796 [0.648–0.977], p=0.029). In contrast, once a euploid blastocyst was obtained, its reproductive potential appeared comparable regardless of whether it was derived from a high or low P/fol environment, as reflected by no significant differences in clinical pregnancy or ongoing pregnancy rates.

Where, then, is progesterone in the late follicular phase produced during ovarian stimulation? This phenomenon has traditionally been explained as "early luteinization" [17]. Luteinization is defined in the Oxford Concise Medical Dictionary as "the process by which follicular cells (granulosa and theca cells) of the ovary are transformed into luteal cells after ovulation, under the influence of luteinizing hormone (LH)." However, even when serum progesterone rises during ovarian stimulation, clinicians commonly observe that, with appropriate final oocyte maturation triggering and oocyte retrieval, mature oocytes surrounded by intact cumulus–oocyte complexes (COCs) can still be obtained. Importantly, these COCs do not show morphological features suggestive of luteinization, such as a dark corona radiata [18]. Taken together, these observations make it difficult to attribute late-follicular progesterone elevation simply to early luteinization. This raises a key question: where is progesterone in the late follicular phase actually produced?

We hypothesize that late–follicular phase progesterone "seeps" from developing follicles into the circulation. It is well established that progesterone is produced in the theca cells within the follicle from circulating cholesterol [19]. Progesterone concentrations in follicular fluid of late–follicular phase developing follicles have been reported to range from 3,000 to 10,000 ng/mL [20], which is approximately 3,000 – 10,000 times higher than serum concentrations. Therefore, when a follicle can no longer retain such a high intrafollicular progesterone level, progesterone may leak from the follicular fluid into the bloodstream (analogous to water seeping from an overfilled plastic bag).

One potential mechanism is a reduction in oocyte-derived factors within follicular fluid [21]. Although many oocyte-secreted factors have been described, we consider growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP-15) to be particularly important because of their putative "anti-luteinization" effects. In other words, follicles with low concentrations of oocyte-derived factors, —especially GDF9 and BMP-15—may represent poor-quality follicles that are unable to maintain high intrafollicular progesterone levels, leading to progesterone seepage into the circulation. Consistent with this concept, lower intrafollicular GDF9/BMP-15 levels have been associated with reduced oocyte competence and poorer embryo development [22], which may ultimately contribute to the reduced euploid rate observed in cycles with elevated P/fol. However, in the present study, clinical pregnancy and ongoing pregnancy rates did not differ between the P/fol-high and P/fol-low groups when euploid blastocysts were transferred. These findings indicate that, even under conditions of elevated P/fol (i.e., increased "progesterone seepage"), once a euploid blastocyst is obtained, we did not observe a statistically significant reduction in pregnancy outcomes after single euploid blastocyst transfer. However, because this study was not designed as an equivalence or non-inferiority study, a modest effect on implantation potential cannot be completely excluded.

Conclusion

In this retrospective PGT-A cohort, elevated late-follicular P/fol was associated with a lower euploid blastocyst rate. In contrast, pregnancy outcomes after single euploid frozen-thawed blastocyst transfer were not significantly different between the P/fol-low and P/fol-high groups. These findings suggest that elevated P/fol may mainly affect the likelihood of obtaining a euploid blastocyst rather than the post-transfer reproductive potential of euploid embryos. Further prospective and external validation studies are warranted.

Acknowledgements

The authors thank medical colleagues and laboratory staff at Sugiyama Clinic Shinjuku for collecting clinical data. English-language editing was assisted by ChatGPT (OpenAI, San Franci) sco, CA, USA). AI tools were not used for study design, statistical analysis, data interpretation, or the peer-review process. The authors alone are responsible for the integrity and accuracy of all content.

Conflicts of interest

Koji Nakagawa, Takashi Horikawa, Keisuke Shiobara, Shota Hatakeyama, Hideaki Watanabe, Satoru Takamizawa, and Rikikazu Sugiyama declare no conflicts of interest that could appear to influence the results from this study.

Funding

This study was conducted with funding from the authors' group without any external sources.

Authors contributions

Nakagawa K is the principal investigator. Horikawa T, Shiobara K, Watanabe H, and Takamizawa S collaborated in the collection of the clinical data. Hatakeyama S and Nakagawa K performed the statistical data analysis. Sugiyama R organized this study. All authors agree with the content of this manuscript. Everyone in the author list reviewed the final manuscript prior to submission.

Human Rights Statements and informed consent

This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards, and it was reviewed and approved by the institutional review board of Sugiyama Clinic (18-0001). All patients received and signed informed written consent forms before entering the study, and they also were given the option to withdraw from the study at any time during treatment.

References

1. Mio Y, Sekijima A, Iwabe T, Onohara Y, Harada T, et al. (1992) Subtle rise in serum progesterone during the follicular phase as a predictor of the outcome of vitro fertilization. Fertil Steril 58: 159-66.
2. Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R (1993) Premature elevation of plasma progesterone alters pregnancy rates of in vitro fertilization and embryo transfer. Fertil Steril. 1993; 59: 1090–94.
3. Shulman A, Ghetler Y, Beyth Y, Ben-Nun I (1996) The significance of an early. (premature) rise of plasma progesterone in in vitro fertilization cycles induced by a "long protocol" of gonadotropin releasing hormone analogue and human menopausal gonadotropins. J Assist Reprod Genet 13: 207–11.
4. Jiang W, LI D, Zhu L, Wang Jie, Chen L, et al. (2022) Elevated serum progesterone levels on the hCG trigger day have a negative impact on the live birth rate in the first fresh IVF-ET cycle. J Obstet Gynaecol 42: 3503-8.
5. Racca A, Santos-Riberio S, Munck ND, Mackens S, Drakopoulos P, et al. (2018) Impact of late－follicular phase elevated serum progesterone on cumulative live birth rates: is there a deleterious effect on embryo? Hum Reprod. 2018; 33: 860-8.
6. Horikawa T, Nakagawa K, Shiobara K, Maruyama T, Takamizawa S, et al. (2026) derived from oocytes retrieved in cycles with elevated late-follicular progesterone per follicle show reduced reproductive potential; an analysis based on progesterone levels per individual follicle. Arch Reprod Med 2: 000528.
7. Sugiyama R, Nakagawa K, Nishi Y, Ojiro Y, Juen H, et al. (2013) Using a mild ovarian stimulation protocol combined with clomiphene citrate and recombinant follicle-stimulation hormone to determine the optimal number of oocytes needed to achieve pregnancy and reduce the concerns patients. Reprod Med Biol 12: 105-10.
8. Nakagawa K, Horikawa T, Shiobara K, Ishiyama S, Kataoka H, et al. (2024) Mild ovarian stimulation using the aromatase inhibitor letrozole (LTZ) is a good stimulation protocol for to obtain euploid blastocysts for advanced maternal age women. J Gynecol Res Obstet 10: 31-39.
9. Nakagawa K, Oba M, Ehara K, Ishigaki N, Ino N, et al. (2018) Clinical outcomes of assisted reproductive technology treatment by using a self-injection of recombinant human chorionic gonadotropin as the final maturation trigger. Reprod Med Biol 17: 203-8.
10. Hiraoka K, Isuge M, Kamada Y, Kaji T, Suhara T, et al. (2021) Kuga A, Ohuchi K, Hayashi M, Kawai K. Piezo-ICSI for human oocytes. J Vis Exp.
11. Nakagawa K, Shirai A, Nishi Y, Sigiyam R, Kuribayashi Y, et al. (2010). A study of the effect of an extremely low oxygen concentration on the development of human embryos in assisted reproductive technology. Reprod Med Biol. 2010; 9: 163-8.
12. Gardner DK, Lane M, Steavens J, Schlenker T, Schoolcraft WB (2000) Blastocyst quality and the prediction of human implantation and pregnancy. Fertil Steril 73: 1155-8.
13. Nakagawa K, Horikawa, Orita Y, Yamashiro E, Watanabe H, Shirai A,et al. (2023) Hyalurinan-enriched transfer medium (HETM) can improve the implantation rate in morphologically poor euploid blastocyst transfer. Arch Gynecol Obstet 308: 611-9.
14. Yang D, Feng D, Gao Y, Sagnelli M, Wang X, et al. (2020) An effective method for trophectoderm biopsy using mechanical blunt dissection: a step-by-step demonstration. Fertil Steril. 2020; 114: 438-9.
15. Sugiyama R, Nakagawa K, Shirai A, Sugiyama R, Nishi Y, et al. (2010) Clinical outcomes resulting from the transfer of vitrified human embryos using a new device fro cryopreservation (plastic blade). J Assist Reprod Genet 27: 161-7.
16. Kanda Y (2013) Investigation of the freely available easy-to-use software "EZR" for medical statistics. Bone Marrow Transplant 48: 452–8.
17. Chou CH, Chen MJ (2018) The effect of steroid hormones in ovarian follicle development. Vitam Horm 107: 155–75.
18. Wang Meng, Xi Q, Li Z, Yang L, Zhu L, et al. (2021) The relationship between a novel evaluation parameter of premature luteinization and IVF outcomes. Reprod BioMed Online 42: 323-1.

19. Hillier, Whitelaw PF (1994) Follicular osetrogen synthesis: the 'two-cell, two-gonadotropin' model revisited. Mol Cell Endocrinol 100: 51-54.

20. Marchianni S, Tamburrino L, Benini B, Pallecchi M, Bignozzi C, et al. (2020) LH supplementation of ovarian stimulation protocols influences follicular fluid steroid composition contributing to the improvement of ovarian response in poor responder women. Scientific Reports 10: 12907.

21. Wang NF, Mamsen LS, Cadenas J, Saritas G, Macklon KT, et al. (2025). Impact of female age on concentrations of reproductive hormones and oocyte-specific growth factors in follicular fluid from human small antral follicles. Hum Reprod. 2025; 40: 707-716.

22. Li Y, Li RQ, Ou SB, Zhang NF, Ren L, et al. (2014) Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. Reprod Biol Endocrinol 12: 81.