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Relationship between a low ratio of serum estradiol to follicle number and fertility treatment outcomes

A retrospective cohort study of 516 cases

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Abstract

The aim of this retrospective study was to examine how a low estradiol/follicle (E2/fol) may be related to in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI)-embryo transfer outcomes in polycystic ovary syndrome (PCOS) and non-PCOS patients, respectively. Between 2013 and 2017, 516 IVF/ICSI cycles (146 cycles in PCOS patients and 370 cycles in non-PCOS patients) with a long gonadotrophin releasing hormone receptor agonist protocol—including 338 involved fresh transfer cycles (89 cycles in PCOS patients and 249 cycles in non-PCOS patients)—were conducted. Outcomes were compared between 5 groups of PCOS patients defined by E2/fol (pg/mL) as follows: A, <140; B, 140 to 210; C, 210 to 280; D, 280 to 350; and E, >350. Non-PCOS patients' outcomes are grouped as well. Whether in PCOS or non-PCOS patients, those in the lowest E2/fol group (<140 pg/mL) tended to be younger, and with a greater body mass index (BMI) and antral follicle count (AFC), than the patients in the other groups. Relative to the other groups, Group A showed a lower number and rate of oocytes, higher single pronucleus (1PN) and triple pronucleus (3PN) formation rate, early and advanced abortion rates, but these did not differ significantly from those of the other groups, it perhaps due to the limited sample size. Group A have a higher incidence of moderate or severe ovarian hyperstimulation syndrome than the other groups in non-PCOS patients ($P > .05$). Whether in PCOS or non-PCOS patients, greater BMI, greater AFC, and younger age may favor the phenomenon of low E2/fol. In turn, low E2/fol may reduce the oocyte retrieval rate and increase the risk of 1PN and 3PN formation and abortion.

Abbreviations: 1PN, 2PN, and 3PN = single, double, and triple pronucleus formation, AFC = antral follicle count, BMI = body mass index, COH = controlled ovarian hyperstimulation, E_2 = estradiol, E_2/fol = estradiol/follicles, FSH = follicle stimulating hormone, Gn = gonadotrophin, GnRH = gonadotrophin releasing hormone, GnRH-a = gonadotrophin releasing hormone receptor agonist, hCG = human chorionic gonadotrophin, IVF/ICSI-ET = in vitro fertilization/intracytoplasmic sperm injection-embryo transfer, LH = luteinizing hormone, OHSS = ovarian hyperstimulation syndrome, P = progesterone, PCOS = polycystic ovary syndrome, PRL = prolactin, T = testosterone.

Keywords: estradiol to follicle ratio, human chorionic gonadotrophin administration, in vitro fertilization/intracytoplasmic sperm injection, polycystic ovary syndrome, treatment outcome

1. Introduction

Infertility is a hot topic in reproductive medicine, with the number of couples affected by infertility increasing worldwide year after year.[1,2] In China, with the shift from a 1-child to a 2-child policy, there has been a surge in older women seeking fertility treatments, including in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI)-embryo transfer (ET). IVF/ICSI-ET requires the harvesting of high-quality eggs following controlled ovarian hyperstimulation (COH),[3–5] a procedure wherein a drug treatment is used to induce follicular development and maturation within a controllable range.

A variety of COH protocols involving different gonadotrophin (Gn) preparations with or without pituitary down-regulation with gonadotrophin releasing hormone (GnRH) agonism or antagonism are available, depending on the individual patient's circumstances.[6] At our center, we prefer to use a long COH protocol owing to its higher success rate. However, a mild stimulation protocol is preferred by some patients. Some studies have shown that the ratio of serum estradiol (E2) level to the

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number of follicles on the day of human chorionic gonadotrophin (hCG) administration, a ratio abbreviated as E2/fol (follicle), provides a useful index for predicting IVF/ICSI-ET outcomes following COH.[7,8] Indeed, some studies have indicated that exceeding the optimal E2/fol range could have negative effects on IVF/ICSI-ET outcome, though the establishment of such an upper bound remains controversial.[7–11] Endometrial receptivity is also considered to be an important factor in IVF/ICSI-ET outcome.[4]

The heterogeneity of patients with polycystic ovary syndrome (PCOS) makes its diagnosis and treatment controversial, but some studies have shown that PCOS is an independent risk factor for the treatment and outcome of IVF/ICSI-ET.

In this study, we analyzed retrospectively IVF/ICSI-ET cycle data for patients with PCOS or non-PCOS treated at our reproductive treatment center between January 2013 and February 2017. Specifically, we sought to elucidate how E2/fol (for follicles ≥ 12 mm in diameter) may be related to patients' clinical baseline data and the treatment regimen employed, as well as how these parameters are related to subsequent embryonic development and pregnancy outcomes following IVF/ICSI-ET.

2. Materials and methods

2.1. Study design and patients

A retrospective review of 516 IVF/ICSI cycles (in 516 patients; 146 cycles in PCOS patients and 370 cycles in non-PCOS patients) undergoing a long gonadotrophin releasing hormone receptor agonist (GnRH-a) protocol for COH was used, of which 338 involved fresh embryo transfers (89 cycles in PCOS patients and 249 cycles in non-PCOS patients) in women with a normal ovarian reserve between January 2013 and February 2017 at the Reproductive Medicine Center of the Affiliated Hospital of Guangdong Medical University. The inclusion criteria were the age of female varies from 20 to 44 years old; or 1 year ≤ duration of infertility ≤ 20 years. The exclusion criteria were women's uterus does not have pregnancy function or they have serious physical illness which cannot afford pregnancy; or incomplete outpatient data. Diagnosis of PCOS based on Rotterdam's standards established in 2003.

This study was approved by the Institutional Review Ethics Committee of Affiliated Hospital of Guangdong Medical University. All patients whose data were included in our analysis provided informed consent for participation before undergoing their treatments. Outcomes were compared across the following 5 E2/fol groups (pg/mL): A, <140; B, 140 to 210; C, 210 to 280; D, 280 to 350; and E, >350. Cases in which E2/fol was <70 pg/mL were included in the A group, rather than forming a separate group, as in Sandoval et al,[12] because such a group would have included fewer than 10 cases.

2.2. Stimulation protocol

All patients were treated with a long GnRH-a rotocol. Briefly, 5 to 7 days after ovulation or after 15 days of being on pre-IVF Marvelon (N.V. Organon, Netherlands), GnRH-a (single dose of 0.8–1.875 mg; Decapepty, Ferring, Germany) was administered. The GnRH-a administration phase ended on the hCG administration day. The Gn phase of therapy, which was started 14 days after starting GnRH-a delivery and was culminated on the hCG injection day, included a natural or synthetic follicle stimulating hormone (FSH) preparation (dose Gonal-F, Merck Serono, Germany; dose Urofollitropin, Livzon Pharmaceutical Group Inc, China; or dose Puregon, Merck Sharp & Dohme) (according to the current regulations of our center, ≤30 years old, starting dose of FSH is 75–150 IU; 31–34 years old starting dose of FSH is 150–225 IU;≥ 35 years old, starting dose of FSH is 225 IU, then according to monitored follicular development to adjust the dose), a luteinizing hormone (LH) preparation (dose Luveris, Merck Serono, Germany), and, finally, hCG (5000–10,000 IU, Livzon Pharmaceutical Group Inc, China) (generally for patients with age ≥ 35 years old, or poor ovarian response will supplement LH, starting dose of LH is 75 IU). Eggs were harvested 34 to 36 hours after hCG injection (generally, a single follicle with a diameter of up to 19 mm, 2 follicles up to 18 mm in diameter, or 3 follicles up to 17 mm in diameter), during which time E2 levels were monitored (generally reaching an average of 250–300 ng/L per dominant follicle [≥16 mm], and a proportion of dominant follicles as high as 60%) (Fig. 1).

2.3. Measurement of serum hormones

Hormone levels were determined in venous blood by the electrochemical method with a cobas 601 analyzer (Roche, Switzerland) in our hospital laboratory. Before beginning an ovulation-

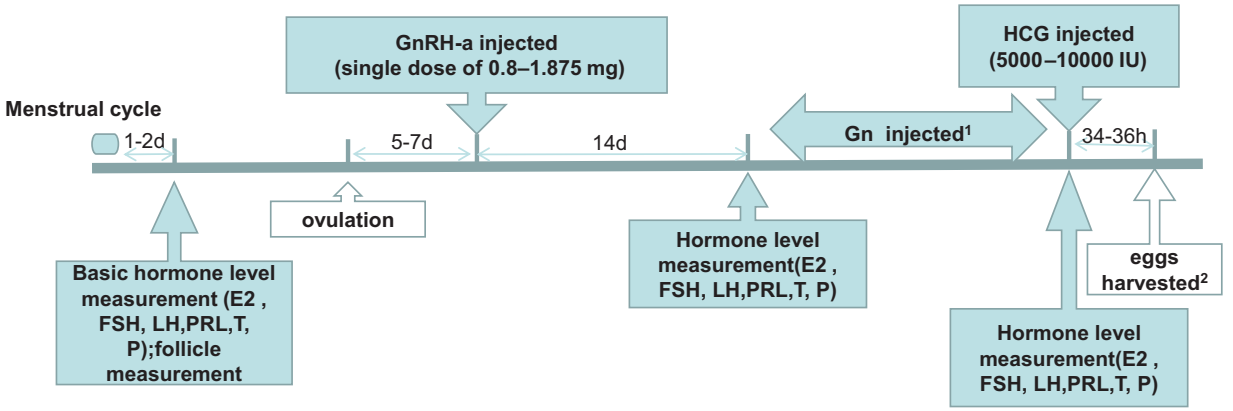

Figure 1. Long gonadotrophin-releasing hormone agonist (GnRH-a) protocol and the time of serum hormones measurement. 1. Gn included natural or synthetic follicle stimulating hormone (FSH) and luteinizing hormone (LH). 2. Time of hCG injected: a single follicle with a diameter of up to 19 mm; 2 follicles up to 18 mm in diameter; 3 follicles up to 17 mm in diameter, during which time E2 levels were monitored (generally reaching an average of 250–300 ng/L per dominant follicle [≥16 mm]); a proportion of dominant follicles (≥16 mm) as high as 60%. hCG = human chorionic gonadotrophin.

stimulating protocol, basal levels of the following hormones were determined in blood taken on day 2 or 3 of the menstrual cycle (follicular phase: reference ranges): E2 (12.4–233.0 pg/mL), FSH (3.5–12.5 IU/L), LH (2.4–12.6 IU/L), prolactin (PRL; 4.79–23.3 ng/mL), testosterone (T; 0–0.8 ng/mL), and progesterone (P; 0.2–1.5 ng/mL). FSH, LH, and E2 levels were re-assessed immediately before starting the Gn phase. LH and P levels were re-assessed immediately before hCG administration (Fig. 1).

2.4. Follicle measurement

A transvaginal ultrasound diagnostic apparatus (Aloka, Japan) with a probe frequency of 5 MHz was used with the patient in the lithotomy position. The vaginal probe was covered with a condom and external lubricant. For each observed follicle, the average of 2 maximum follicle cross-sectional diameters was recorded. The antral follicle count (AFC), that is, the number of bilateral ovarian follicles with a diameter in the range of 2 to 10 mm during menstrual cycle days 1 to 3 was determined. The number of follicles $\geq$ 12 mm in diameter that was present bilaterally on the hCG trigger day was also determined (Fig. 1).

2.5. Observed parameters

Baseline data included patient age, duration of infertility, body mass index (BMI), AFC, and basal hormone data (E2, FSH, LH, P, PRL, and T). COH indicator data included GnRH-a dose, Gn phase commencement hormone levels (FSH, LH, and E2; Gn phase levels were compared to basal hormone data to determine hormone decline), FSH starting dose, duration of FSH, FSH total dose, LH total dose, and hormone (LH and P) levels on the day of hCG administration.

The following oocyte and embryo parameters were analyzed: oocyte retrieval rate; number of oocytes retrieved; egg maturation rate; total fertilization rate; single, double, and triple pronucleus formation (1PN, 2PN, and 3PN, respectively) rates, 2PN cleavage rate, number and rate of high-quality embryos (7–9 cells, grade I), and cleavage-stage embryo utilization rate. In terms of complications, we recorded the incidence of moderate or severe ovarian hyperstimulation syndrome (OHSS). Finally, we tracked the following indicators of pregnancy outcome: implantation rate, nonpregnancy rate, biochemical pregnancy rate, clinical pregnancy rate, early abortion rate (before 12 weeks gestation), advanced abortion rate (12–28 weeks gestation), and twin/multiple pregnancy rate.

2.6. Statistical analysis

The Shapiro–Wilks test was used to evaluate data distributions. For continuous variables, normally distributed datasets are reported as means with standard deviations (SDs) and nonparametric datasets are presented as medians with ranges. Variance among mean values was determined with 1-way analyses of variance. Post hoc intergroup comparisons for performed with Bonferroni method if there was variance homogeneity. Otherwise, Games–Howell tests were performed. Differences between proportions were evaluated with the chi-squared test or Fisher exact test. Comparisons were considered statistically significant at $P < .05$.

3. Results

3.1. Baseline data for patients

To exclude the effect of PCOS, the relationship between each index and E2/fol in PCOS patients and non-PCOS patients will be analyzed separately. The major subset of 146 PCOS patients had a mean age of 29.1 ± 3.8 years, a mean BMI of 22.0 ± 3.6 kg/m^2, a mean AFC of 26.3 ± 12.1, and the following basal hormone levels: E2, 54.6 ± 48.2 pg/L; FSH, 6.3 ± 1.6 IU/L; LH, 8.1 ± 4.2 IU/L; PRL, 18.4 ± 10.1 ng/L; P, 0.7 ± 0.8 ng/L; and T, 0.5 ± 0.6 ng. In 370 non-PCOS patients, they had a mean age of 30.9 ± 4.4 years, a mean BMI of 21.0 ± 2.5 kg/m^2, a mean AFC of 18.1 ± 7.7, and the following basal hormone levels: E2, 52.1 ± 56.2 pg/L; FSH, 6.8 ± 1.7 IU/L; LH, 5.3 ± 2.2 IU/L; PRL, 24.1 ± 19.4 ng/L; P, 0.7 ± 0.6 ng/L; and T, 0.3 ± 0.4 ng. The distribution is shown in Table 1.

3.2. Comparison of clinical baseline data across E2/fol groups

All clinical baseline data for the E2/Fol groups are reported in Table 2 together with comparison *P* values. Whether it is a PCOS patient or not, the women in the lowest E2/fol group (A: <140 pg/mL) were, on average, younger than the other 4 groups of women ($P < .05$). Mean BMI was significantly higher in group A than the other 4 groups ($P < .05$). AFC in PCOS patients tended to decrease with increasing E2/fol (except group C), and the AFC for group A was significantly greater than the AFC for the other 4 groups ($P < .05$). In non-PCOS patients, lowest and highest E2/fol group both have more AFC reserves than the other 3 groups ($P < .05$). Mean basal E2 levels tended to higher in group A, but

Table 1

Distribution of E2/fol groups in the patient with or without PCOS (PCOS, n = 146; non-PCOS, n = 370).

	E2/fol group, pg/mL				
Baseline variables	**A (<140)**	**B (140–210)**	**C (210–280)**	**D (280–350)**	**E (>350)**
Total IVF/ICSI cycles (n = 516)					
PCOS (N = 146)	43 (29.5%)	47 (32.2%)	25 (17.1%)	15 (10.3%)	16 (11.0%)
Non-PCOS (N = 370)	57 (15.4%)	117 (31.6%)	83 (22.4%)	43 (11.6%)	70 (18.9%)
Total	100	164	108	58	86
Fresh transfer cycles (n = 338)					
PCOS (N = 89)	31 (34.8%)	34 (38.2%)	13 (14.6%)	7 (7.9%)	4 (4.5%)
Non-PCOS (N = 249)	41 (16.5%)	89 (35.7%)	63 (25.3%)	27 (10.8%)	29 (11.6%)
Total	72	123	76	34	33

IVF/ICSI = in vitro fertilization/intracytoplasmic sperm injection, PCOS = polycystic ovary syndrome.

Table 2

Comparison clinical baseline data among E2/fol groups within the patient subset (PCOS, n = 146; non-PCOS, n = 370).

Baseline variables	E2/fol group, pg/mL					
	A (<140); PCOS, n = 43 (non-PCOS, n = 57)	B (140–210); PCOS, n = 47 (non-PCOS, n = 117)	C (210–280); PCOS, n = 25 (non-PCOS, n = 83)	D (280–350); PCOS, n = 15 (non-PCOS, n = 43)	E (>350); PCOS, n = 16 (non-PCOS, n = 70)	$P^{\dagger}$
Age, y	28.1 ± 3.6 (30.2 ± 4.1)	29.1 ± 3.8 (31.2 ± 4.4)	28.5 ± 3.8 (31.5 ± 4.6)	31.6 ± 3.2* (30.7 ± 4.9)	30.0 ± 3.4 (30.2 ± 4.0)	<.05‡ (<.05§)
Duration of infertility, y	3.6 ± 1.7 (3.3 ± 2.3)	3.9 ± 2.0 (3.8 ± 3.2)	3.0 ± 2.2 (3.7 ± 2.8)	2.7 ± 1.7 (3.9 ± 2.6)	3.8 ± 2.7 (2.8 ± 1.9)	>.05‡ (>.05§)
BMI, kg/m^2	23.2 ± 4.5 (22.0 ± 2.4)	22.2 ± 3.1 (21.3 ± 2.8)	20.1 ± 3.3 (20.9 ± 2.2)	21.7 ± 2.5 (20.2 ± 2.2*)	20.2 ± 2.5* (20.3 ± 2.2*)	<.05‡ (<.05§)
AFC	29.7 ± 15.2 (22.3 ± 11.8)	25.13 ± 9.8 (16.0 ± 5.3*)	25.40 ± 12.3 (17.0 ± 7.3)	24.6 ± 11.4 (16.6 ± 6.5)	23.7 ± 7.7 (20.2 ± 6.4)	>.05‡ (<.05§)
Basal hormone levels						
E2, pg/L	60.4 ± 60.8 (55.6 ± 88.2)	54.9 ± 52.4 (51.7 ± 50.0)	51.7 ± 31.3 (49.6 ± 45.8)	40.9 ± 14.9 (59.1 ± 59.9)	55.8 ± 39.7 (48.8 ± 40.8)	>.05‡ (>.05‡)
FSH, IU/L	5.8 ± 1.6 (6.5 ± 1.6)	6.5 ± 1.5 (6.8 ± 1.8)	6.5 ± 1.6 (7.1 ± 1.8)	6.4 ± 1.6 (7.1 ± 1.5)	6.0 ± 1.2 (6.6 ± 1.5)	>.05‡ (>.05‡)
LH, IU/L	7.8 ± 3.9 (5.2 ± 2.6)	8.3 ± 4.1 (5.0 ± 2.2)	7.7 ± 4.3 (5.1 ± 1.9)	9.0 ± 5.7 (5.6 ± 2.2)	8.0 ± 3.3 (6.1 ± 2.2)	>.05‡ (<.05‡)
PRL, ng/L	16.2 ± 9.1 (16.2 ± 9.1)	18.7 ± 10.4 (18.7 ± 10.4)	17.3 ± 9.2 (17.3 ± 9.2)	22.2 ± 12.3 (22.2 ± 12.3)	21.7 ± 10.2 (21.7 ± 10.2)	>.05‡ (>.05‡)
P, ng/L	0.7 ± 0.7 (0.6 ± 0.8)	0.7 ± 0.9 (0.7 ± 1.1)	0.5 ± 0.8 (0.5 ± 0.4)	0.6 ± 0.5 (0.5 ± 0.5)	0.8 ± 0.9 (0.6 ± 0.8)	>.05‡ (>.05‡)
T, ng/L	0.6 ± 0.8 (0.3 ± 0.4)	0.4 ± 0.6 (0.4 ± 0.6)	0.3 ± 0.2* (0.3 ± 0.4)	0.4 ± 0.2 (0.2 ± 0.2)	0.3 ± 0.2* (0.3 ± 0.2)	>.05§ (>.05§)
FSH/LH	0.9 ± 0.4 (1.4 ± 0.5)	1.0 ± 0.5 (1.6 ± 0.8)	1.2 ± 0.8 (1.6 ± 0.8)	1.0 ± 0.7 (1.4 ± 0.6)	0.9 ± 0.5 (1.2 ± 0.4)	>.05‡ (<.05§)

Statistics of non-PCOS patients are in parentheses.

AFC = antral follicle count, BMI = body mass index, E_2/fol = estradiol/follicles, FSH = follicle stimulating hormone, LH = luteinizing hormone, P = progesterone, PCOS = polycystic ovary syndrome, PRL = prolactin, T = testosterone.

† One-way analyses of variance.

‡ Bonferroni post hoc tests performed.

§ Games–Howell test performed.

* $P < .05$ for group A vs other groups.

level of the other hormone in group A seems to similar to other groups, but there were no significant differences.

These results suggest that age, BMI, and AFC may be related to a low E2/Fol. Younger women and women with a larger BMI or greater AFC may be prone to a low E2/fol in ART.

3.3. Gn phase parameters following pituitary down regulation: COH indicator data comparison across E2/fol groups

GnRH-a dosage was similar across groups A, B, C, and E, though that in Group D was significantly lower than that in Group A ($P > .05$). After pituitary down-regulating GnRH-a treatment, FSH, E2, and LH levels measured immediately before Gn treatment were decreased relative to basal levels. However, there was no statistically significant difference in the decrease in hormones levels between groups. Follow-up Gn dose and time of use did not differ significantly between groups. In PCOS patients, group B (E2/fol, 140–210 pg/mL) had the higher mean total FSH and LH dosage. Group D (E2/fol, 280–350 pg/mL) had a lowest mean total FSH dosage. Group D in non-PCOS patients had opposite trend in mean total FSH dosage. Lower LH dosage had a lower clinical pregnancy rate (group B).

As shown in Table 3, mean levels of LH ($P < .05$) and P ($P < .05$) on the hCG injection day differed significantly across the E2/fol groups, with group A having lower levels of both hormones than groups B, C, D, and E.

3.4. Comparisons of oocyte and embryo quality and moderate or severe OHSS incidence among E2/fol groups

Oocyte and embryo parameter data for the E2/fol groups of patients who completed the long GnRH-a protocol for COH (PCOS, n = 146; non-PCOS, n = 370) are reported and compared in Table 4. The oocyte retrieval rate differed significantly across the E_2/fol groups, with group A having a lower rate than the other 4 groups. The number of oocytes retrieved was more in the groups of lower E2/fol (group A) and higher E2/fol (groups D and E) non-PCOS patients ($P < .05$). But it was lowest in the groups of lower E_2/fol (group A) in PCOS patients, and increased with increasing E2/fol (E2/fol < 350 pg/mL) ($P > .05$). In PCOS patients, the 1PN formation rate for group A was intermediate between groups for non-PCOS patients ($P < .05$), but it was significantly higher than that of groups C, D, and E ($P > .05$). The 3PN formation rate was highest in group A regardless of PCOS or non-PCOS patients ($P > .05$). Total fertilization rate and 2PN formation and cleavage rates in group A were intermediate between groups ($P > .05$). Meanwhile, high-quality embryo number and rate, and cleavage-stage embryo utilization rate were statistically similar across the 5 groups.

As is shown in Table 4, in non-PCOS patients, the incidence of moderate-to-severe OHSS in lower E2/fol (group A) was highest among the groups ($P < .05$), but group A and B was lower than that of higher E2/fol (groups C, D, and E for PCOS patients ($P > .05$).

3.5. Comparisons of pregnancy outcomes among E2/fol groups with fresh embryo transfer cycles

Pregnancy outcome parameters did not differ significantly across the E2/fol groups (Table 5). However, it is worth noting that, although not significantly so, group A had higher early- and late-stage abortion rates regardless of PCOS or non-PCOS patients, and group E had highest early-stage abortion rates. Group B has highest implantation rate and clinical pregnancy rate with high biochemical pregnancy rates in PCOS patients; while higher implantation rate and clinical pregnancy rate were shown in group D in non-PCOS patients (Table 5).

4. Discussion

In the present study, we found that patients with E2/fol < 140 pg/mL tended to be younger, and with a greater BMI and AFC, than patients with a higher E2/fol. The E2/fol < 140 pg/mL group had lowest LH and P levels on the day of hCG administration, a lower number (in PCOS patients) and rate of oocytes, and a higher 3PN

Table 3

Comparison of pre-Gn treatment variables among E2/fol groups after pituitary down-regulation (PCOS, n = 146; non-PCOS, n = 370).

	E2/fol group, pg/mL					
Gn phase parameters	**A (<140); PCOS, n = 43 (non-PCOS, n = 57)**	**B (140–210); PCOS, n = 47 (non-PCOS, n = 117)**	**C (210–280); PCOS, n = 25 (non-PCOS, n = 83)**	**D (280–350); PCOS, n = 15 (non-PCOS, n = 43)**	**E (>350); PCOS, n = 16 (non-PCOS, n = 70)**	***P*†**
GnRH-a dose, mg	1.2 ± 0.3 (1.1 ± 0.3)	1.1 ± 0.2 (1.1 ± 0.3)	1.2 ± 0.3 (1.1 ± 0.3)	1.0 ± 0.1* (1.0 ± 0.1)	1.2 ± 0.4 (1.0 ± 0.2)	>.05‡ (>.05‡)
Level on Gn start day						
E2, pg/L	9.3 ± 8.4 (8.2 ± 7.5)	9.1 ± 7.8 (8.5 ± 7.3)	9.2 ± 6.3 (11.1 ± 9.5)	5.8 ± 2.9 (9.4 ± 7.5)	11.7 ± 13.2 (7.6 ± 7.7)	>.05‡ (>.05‡)
FSH, IU/L	2.6 ± 0.9 (2.4 ± 1.2)	2.2 ± 0.9 (2.4 ± 1.3)	2.5 ± 1.1 (2.3 ± 1.3)	2.0 ± 0.6 (2.4 ± 1.3)	2.2 ± 1.3 (1.9 ± 0.9)	>.05§ (<.05§)
LH, IU/L	1.7 ± 0.6 (1.6 ± 0.5)	1.9 ± 0.7 (1.9 ± 0.7*)	1.9 ± 0.7 (1.9 ± 0.7*)	1.8 ± 0.6 (2.1 ± 1.0*)	1.7 ± 0.9 (2.1 ± 0.8*)	>.05§ (<.05‡)
E2 decline, pg/L	51.0 ± 62.4 (47.4 ± 89.0)	45.8 ± 53.5 (43.2 ± 50.0)	42.5 ± 33.1 (38.6 ± 46.3)	35.1 ± 14.7 (49.8 ± 61.4)	42.2 ± 28.5 (41.2 ± 40.7)	>.05§ (>.05§)
FSH decline, IU/L	3.3 ± 1.7 (4.1 ± 1.8)	4.3 ± 1.8 (4.4 ± 2.0)	4.1 ± 2.1 (4.9 ± 1.9)	4.4 ± 1.7 (4.7 ± 1.8)	3.8 ± 1.7 (4.7 ± 1.7)	<.05§ (>.05§)
LH decline, IU/L	6.1 ± 3.9 (3.6 ± 2.7)	6.4 ± 4.1 (3.2 ± 2.3)	5.8 ± 4.5 (3.2 ± 1.9)	7.2 ± 5.6 (3.5 ± 2.3)	6.3 ± 3.5 (4.0 ± 2.1)	>.05§ (>.05§)
FSH starting dose, IU	153.5 ± 41.4 (187.1 ± 47.8)	164.4 ± 46.4 (183.1 ± 57.2)	147.5 ± 52.0 (199.4 ± 55.0)	178.3 ± 45.2 (169.2 ± 54.4)	159.4 ± 46.7 (179.1 ± 44.7)	>.05§ (>.05§)
Duration of Gn, d	11.5 ± 3.1 (11.0 ± 1.9)	10.9 ± 2.1 (10.9 ± 1.7)	10.8 ± 2.0 (10.8 ± 1.9)	10.0 ± 2.7 (11.2 ± 1.2)	11.3 ± 1.7 (10.6 ± 1.3)	>.05§ (>.05§)
FSH total dose, IU	1942.7 ± 896.8 (2225.4 ± 510.1)	1964.9 ± 738.9 (2246.8 ± 660.4)	1877.0 ± 833.6 (2427.7 ± 763.7)	1774.2 ± 727.1 (2256.4 ± 620.1)	1993.8 ± 612.0 (2024.7 ± 637.0)	>.05§ (>.05§)
LH total dose, IU	216.3 ± 275.4 (175.0 ± 299.1)	199.5 ± 238.9 (152.6 ± 226.7)	219.0 ± 301.5 (258.4 ± 299.4)	210.0 ± 269.2 (245.9 ± 277.9)	206.3 ± 330.3 (201.4 ± 241.4)	>.05§ (<.05‡)
LH on hCG day, IU/L	1.0 ± 0.6 (1.1 ± 0.6)	1.5 ± 1.0* (1.3 ± 0.6)	1.4 ± 0.4* (1.5 ± 0.9*)	1.2 ± 0.6 (1.7 ± 1.0*)	1.2 ± 0.4 (1.6 ± 0.9*)	<.05§ (<.05‡)
P on hCG day, ng/L	0.6 ± 0.3 (0.7 ± 0.3)	0.7 ± 0.3 (0.7 ± 0.4)	0.8 ± 0.5 (0.7 ± 0.5)	0.8 ± 0.4 (0.8 ± 0.4)	1.0 ± 0.7 (0.9 ± 0.5*)	<.05‡ (<.05§)

Statistics of non-PCOS patients are in parentheses.

E_2/fol = estradiol/follicles, FSH = follicle stimulating hormone, Gn = gonadotrophin, GnRH-a = gonadotrophin releasing hormone receptor agonist, hCG = human chorionic gonadotrophin, LH = luteinizing hormone, PCOS = polycystic ovary syndrome.

† One-way analyses of variance.

‡ Games–Howell test performed.

§ Bonferroni post hoc tests performed.

* $P < .05$ for group A vs other groups.

formation rate, incidence of moderate/severe OHSS(in non-PCOS patients), and abortion rate than the 4 higher E2/fol groups.

Childbirth is the outcome of IVF/ICSI-ET that is the common aspiration of doctors and patients. The success of IVF/ICSI-ET depends on the interplay of a number of factors, including effective COH therapy, collection of quality embryos, embryo culture conditions, and full transformation of the endometrium with appropriate endometrial receptivity, which depends on the individual patient's condition.[4,5,7,12]

E2 levels have been suggested to be a key predictor of follicle and endometrial development.[13–15] Loumaye et al[16] proposed E2/fol as a predictor of IVF success, and this suggestion was supported by subsequent studies by other groups.[7,8] Notably, when Triwitayakorn et al[17] divided follicles into 3 groups according to their diameters (<10, 10–14, and >14 mm), they

Table 4

Comparison of oocyte and embryo quality among E2/fol groups after pituitary down-regulation (PCOS, n = 146; non-PCOS, n = 370).

	E2/fol group, pg/mL					
Outcomes	**A (<140); PCOS, n = 43 (non-PCOS, n = 57)**	**B (140–210); PCOS, n = 47 (non-PCOS, n = 117)**	**C (210–280); PCOS, n = 25 (non-PCOS, n = 83)**	**D (280–350); PCOS, n = 15 (non-PCOS, n = 43)**	**E (>350); PCOS, n = 16 (non-PCOS, n = 70)**	***P*†**
Oocyte retrieval rate, %	73.1 (82.1)	82.6 (84.9)	86.6 (87.7)	92.1 (89.8)	78.7 (90.3*)	<.05 (>.05)
No. of oocyte retrieved	13.8 ± 6.1 (14.5 ± 5.7)	14.1 ± 6.1 (12.7 ± 6.2)	17.2 ± 9.8 (12.1 ± 6.2)	20.8 ± 8.0 (14.4 ± 7.1)	18.4 ± 7.6* (18.7 ± 7.7)	>.05‡,§ (<.05‡,§)
Egg maturation rate, %	86.7 (84.8)	80.1 (87.3)	81.7 (85.1)	78.5 (86.9)	91.7 (83.6)	>.05 (>.05)
Total fertilization rate, %	77.2 (76.2)	70.6 (81.0)	69.1 (76.1)	71.3 (77.8)	80.4 (74.7)	>.05 (>.05)
1PN formation rate, %	4.5 (2.9)	5.5 (3.8)	2.4 (5.6)	2.6 (5.2)	3.4 (2.4)	>.05 (<.05)
2PN formation rate, %	64.5 (65.2)	60.0 (70.3)	62.3 (64.3)	64.6 (66.3)	73.0 (65.5)	<.05 (>.05)
3PN formation rate, %	4.3 (6.1)	3.2 (4.4)	3.3 (3.5)	2.7* (3.3)	3.0 (3.8)	>.05 (>.05)
2PN cleavage rate, %	97.2 (91.6)	94.8 (97.0)	93.1 (94.6)	97.5 (98.2)	97.6 (95.6)	<.05 (>.05)
No. of high-quality embryos	3.8 ± 2.7 (3.5 ± 3.0)	3.0 ± 2.9 (3.0 ± 2.9)	5.0 ± 3.8 (2.6 ± 2.3)	5.5 ± 4.0 (2.8 ± 2.3)	3.8 ± 2.7 (4.5 ± 4.3)	>.05 (>.05)
Rate of high-quality embryos, %	40.9 (35.7)	33.5 (32.3)	43.5 (35.2)	38.5 (31.4)	28.1 (35.5)	>.05 (>.05)
Cleavage-stage embryo utilization rate, %	67.2 (57.4)	62.0 (58.2)	56.1 (61.4)	46.8* (57.9)	58.5 (55.1)	>.05§ (>.05‡)
Incidence of moderate to severe OHSS, %	9.3 (10.5)	8.5 (0.0*)	20.0* (0.0*)	13.3 (4.7)	18.8 (4.3)	>.05 (>.05)

Statistics of non-PCOS patients are in parentheses.

1PN, 2PN, and 3PN = single, double, and triple pronucleus formation, E_2/fol = estradiol/follicles, OHSS = ovarian hyperstimulation syndrome , PCOS = polycystic ovary syndrome.

† Chi-squared test or Fisher exact test.

‡ One-way analyses of variance.

§ Games–Howell test performed.

* $P < .05$ for group A vs other groups.

Table 5

Comparison of complication and pregnancy outcomes among E2/fol groups of patients followed by fresh embryo transfer (PCOS, n = 89; non-PCOS, n = 249).

Outcomes	E2/fol group, pg/mL					P
	A (<140); PCOS, n = 31 (non-PCOS, n = 41)	B (140–210); PCOS, n = 34 (non-PCOS, n = 89)	C (210–280); PCOS, n = 13 (non-PCOS, n = 63)	D (280–350); PCOS, n = 7 (non-PCOS, n = 27)	E (>350); PCOS, n = 4 (non-PCOS, n = 29)	
Implantation rate, %	40.3 (40.2)	44.6 (39.9)	30.8 (35.7)	42.9 (45.1)	37.5 (48.3)	>.05 (>.05)
Nonpregnancy rate, %	35.5 (29.3)	17.6 (38.2)	46.2 (34.9)	42.9 (14.8)	25.0 (34.5)	>.05 (>.05)
Biochemical pregnancy rate, %	9.7 (9.7)	17.6 (6.7)	7.6 (6.3)	14.2 (14.8)	25.0 (3.4)	>.05 (>.05)
Clinical pregnancy rate, %	54.8 (61.0)	64.8 (55.1)	46.2 (58.8)	42.9 (70.4)	50.0 (62.1)	>.05 (>.05)
Early abortion rate, %	6.5 (7.3)	0.0 (5.6)	0.0 (4.8)	0.0 (3.7)	25 (10.3)	>.05 (>.05)
Advanced abortion rate, %	3.2 (4.9)	2.9 (0.0)	0.0 (1.6)	0.0 (0.0)	0.0 (0.0)	>.05 (>.05)

P for group A vs other groups. Differences between proportions were evaluated with the chi-squared test or Fisher exact test. Statistics of non-PCOS patients are in parentheses.
E_2/fol = estradiol/follicles, PCOS = polycystic ovary syndrome.

found that although the fertilization rates and numbers of high-quality embryos obtained were similar across the 3 groups, a larger diameter follicle facilitated oocyte recovery. Akbariasbagh et al[18] found that the size of follicles and the oocytes maturity in stimulated ovaries are not considerably associated, thus might be independent factors. There was a significant difference between the diameter > 21 mm group and diameter < 12 mm group regarding oocyte maturity and collection rate of metaphase II oocyte. It has been suggested that follicles that are at least 12 mm in diameter have favorable fertilization and cleavage rates.[19] In our clinical experience, we have also found that having follicles with a diameter >12 mm on hCG day tends to lead to successful oocyte harvests, fertilization, and embryo formation. Dickey et al[20] reported that triplet and higher-order implantations were associated positively with the number of follicles with a diameter ≥ 12 mm (but not >18 mm), maternal age, and E2 levels in cycles of human menopausal Gn, with and without clomiphene. In our study with 5 groups based on E2/fol (for follicles ≥ 12 mm in diameter), we found that the long GnRH-a protocol commonly resulted in E2/fol < 140 pg/mL.

As it is well known that both age and BMI can affect the fertility. Sneed et al[21] demonstrated that a higher BMI has a pronounced negative influence on pregnancy rate at younger ages, but this effect is attenuated as age increased. But Chen et al[22] found that age, but not the BMI, had significant effects on IVF/ICSI treatment and infer that losing weight before IVF or ICSI treatment is effective in reducing the dose of Gn. There is no study of relationship between age or BMI and E2/fol. In our study, lower E2/fol tended to be younger, and with a greater BMI, we infer that increased BMI results in poor reactivity of the same amount of Gn and the granule cell secretes E2 reduction. Lower E2/fol group have a lower LH level on hCG day may also be one of the reasons for the decrease of E2 secretion of follicular granule cells.

AFC has been suggested by some to be a more accurate predictor of IVF success than age or ovarian reserve.[23,24] Notably, Fleming et al[25] found that AFC was a good indicator of ovarian response. Here, we found that younger maternal age, a greater AFC, and a greater BMI after pituitary degeneration were associated with a low E2/fol. Var et al[26] suggested that elevated E2 and LH levels on the day that Gn administration was started were associated with excessive regulation before Gn. Our data showed that there was no statistically significant difference in the change of hormone levels at the Gn administration day between groups. Ozdegirmenci et al[8] found that E2/fol was associated with egg number, number of mature eggs, and number of fertilized eggs, but not associated with clinical pregnancy rate. In a study of 342 IVF cycles, Mittal et al[7] found that E2/fol correlated positively with the number of mature eggs and the number of fertilized eggs, regardless of cleavage rate. Vaughan et al[10] found that patients with a low E2/fol ratio tended to have a greater number of mature oocytes. Var et al[26] and Khalaf et al[27] found that a lower E2/fol was associated with a worse pregnancy rate.

LH and P levels on the day of hCG administration (after Gn treatment) were significantly lower in our lowest E2/fol group (group A) than in the other groups. Dirnfeld et al[28] suggested that a very modest increase in serum P levels on the day of hCG administration may be disruptive to conception and successful pregnancy when they found that the pregnancy rate per embryo transfer was 53% (15/28) in patients with P < 0.6 ng/L on the hCG administration day but only 10% (8/80) in patients with P > 0.6 ng/L on the hCG administration day. Serum LH levels in the late follicular phase should be at least 1.2 IU/L to support follicular development and high-quality retrieved oocytes.[29] Meanwhile, E2 levels during this time window appear to not affect endometrial health.[13] In our study, the E2/fol < 140 pg/mL group had the highest 3PN formation rate, higher rates of early and advanced abortion, and implantation and clinical pregnancy rates were not as high as other groups. We suspect that the dosage of LH and P is not sufficient to maintain a subsequent pregnancy, resulting in miscarriage.

Because E2 is secreted predominantly by granulosa cells in follicles,[30] low E2/fol may be suggestive of poor growth of these granulosa cells. Low E2/fol may lead the clinician to delay hCG injection, which may affect the egg retrieval rate. Rittenberg et al[31] suggested that a high BMI may increase abortion rates, whereas Wei et al[32] found that low LH and E2 levels on the day of hCG administration had a negative influence on clinical pregnancy rate, while low P was inconsequential. Santos-Ribeiro et al[33] suggested successful IVF outcomes would be limited by P levels that were too low or too high. Here, in this study, we found that patients with low E2/fol tended to have a higher BMI and lower serum levels of sex hormones, which may lead to greater early abortion risk.

Papageorgiou et al[34] and Mittal et al[7] did not find significant effects of high E2 on the quality of oocytes and embryos nor on pregnancy rates. Mittal et al[7] found that serum E2 is an important determinant of IVF success. While total serum E2 does not exert any positive or negative influence on IVF outcome, E2 per mature follicle and retrieved oocytes do have an impact. Pregnancy rate is better when E2/fol is between 200 and 299.99 pg/mL. Also, increasing serum E2/fol positively correlates with better oocytes and embryo quality. By contrast, E2/O negatively

correlates with oocytes and embryo quality parameters. Ozdegirmenci et al[8] found that when the E2/fol exceeded 540 pg/mL, the number of eggs and of mature eggs was greater than in lower E2/fol groups, albeit not significantly so, and that the clinical pregnancy rate increased. Hu et al[35] found that E2/fol affected implantation rate and abortion rate in patients <35 years of age, with an increased implantation rate being observed with E2/fol in the 279.83 to 552.28 pg/mL range and an increased abortion rate being observed with E2/fol > 552.28 pg/mL. In our study, with a greater E2/fol, especially >350 pg/mL, we tended to see a greater number and rate of eggs retrieved, a greater implantation rate, and a greater early abortion rate and pregnancy rate in non-PCOS patients, group A had a greater abortion rate, though not with statistical significance perhaps due to the limited sample size. But in PCOS patients, group B had shown a highest implantation rate and pregnancy rate, while it has a higher advanced abortion rate (the highest abortion rate was in group A). Large Gn dosages have been shown to decrease egg and embryo quality.[36] However, Labarta et al[37] observed that the more oocytes retrieved, the more euploid embryos are obtained. In our study, in PCOS patients, group B (E2/fol, 140–210 pg/mL) had the higher mean total FSH and LH dosage, but we did not see evidence that the Gn exposure in this group disrupted outcomes in terms of fertilization or 2PN formation, but it had a higher implantation and clinical pregnancy rates. Group D (E2/fol, 280–350 pg/mL) had a lowest mean total FSH dosage and lower fertilization or 2PN formation and clinical pregnancy rate. We suppose that low-dose Gn has greater effect on fertilization rate and clinical pregnancy rate in patients with PCOS. Group D in non-PCOS patients had opposite trend in mean total FSH dosage, fertilization or 2PN formation and clinical pregnancy rate. Lower LH dosage had a lower clinical pregnancy rate (group B). These data indicate that the LH dosage is closely related to the clinical pregnancy rate.

This study has a number of limitations that should be acknowledged. First, this was a retrospective analysis. Second, a major limitation of our study was its relatively small sample size which may have prevented statistical detection of clinically significant differences. Third, we did not analyze pregnancy outcomes of the remaining cryopreserved embryos after transplantation. Finally, due to the limited follow-up period, we did not include some important indicators, such as live birth rate, congenital deformity rate, and infant development.

5. Conclusions

In summary, patients, regardless of PCOS or non-PCOS patients, who are younger, have a greater AFC, or have a greater BMI appear to be more prone to a low E2/fol (<140 pg/mL). Thus, their physicians should be alert to phenomenon of low E2/fol, which may increase risks for of 3PN formation and abortion, and inform the patients in advance of the occurrence and precautions for miscarriage. Elucidating the potential relationship of a low E2/fol with high-quality embryo rate, implantation rate, clinical pregnancy rate, and abortion rate will require further study with larger population samples.

Author contributions

Qiaoyao Huang: Protocol/project development, data analysis, and manuscript writing. Yanru Niu, Bi Chen, Li Jun Song, Lihua Xu, and Xia Jing: Data collection. Yunshan Zhang: Data analysis. Tianzhong Ma: Manuscript reviewing.

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