Effect of large follicle puncture on IVF-ET outcome in patients with unsynchronized follicle maturationcan

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Abstract

Objective: This retrospective study was conducted to explore causes of unsynchronized follicular maturation (UFM) and analyze the effects of large follicle puncture on embryo quality and pregnancy outcome.

Methods: Clinical features and controlled ovulation hyperstimulation (COH) were compared between the puncture group (n = 48) and the control group (n = 2545). We analyzed the COH process with *in vitro* fertilization during fresh cycle embryo transfer with different clinical pregnancy outcomes. We compared clinical characteristics and COH process of patients in the clinical pregnancy (n = 774) and non-clinical pregnancy (n = 527) groups. Finally, factors related to pregnancy outcomes were analyzed using multivariate logistic regression analysis.

Results: Age, level of estradiol on down-regulation day, and initial gonadotropin dose were significantly higher in the puncture group than in the control group. We detected significant differences in age, infertility, and body mass index (BMI) between the clinical and non-clinical pregnancy groups. Age, BMI, and endometrial thickness on the day of human chorionic gonadotropin administration were the independent factors influencing pregnancy outcome.

Conclusions: Patient's age and level of anti-Müllerian hormone were the main factors causing UFM in patients undergoing COH. Large follicle puncture had no significant effect on pregnancy outcome.

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Introduction

Since Porter et al.[1] first applied gonadotropin-releasing hormone agonist (GnRH-a) for *in vitro* fertilization (IVF) in 1984, GnRH-a has been widely used to down-regulate the function of the pituitary gland. As one of the most commonly used regimens for controlled ovarian hyperstimulation (COH), GnRH-a has many advantages, including more stable drug concentration, better controllability, improved follicular maturation, fewer early luteinizing hormone (LH) peaks, lower cycle cancellation rate, and higher pregnancy rate. However, unsynchronized follicular maturation (UFM) can occur during this treatment regimen. Several studies have suggested that UFM directly affects embryonic development and pregnancy outcome.[2] Puncture of larger follicles results in aspiration of steroid-rich follicular fluid, reduces circulating levels of steroid hormones, and eliminates endogenous LH peaks that may be induced, thereby eliminating the direct inhibition of adjacent follicles by the dominant follicles, facilitating the synchronized development of the remaining follicles and improving oocyte maturation rate and pregnancy rate. In the present study, we analyzed the clinical characteristics and outcomes of 48 patients undergoing large follicle puncture during a long-term GnRH-a and COH protocol and explored the causes of UFM and the effect of large follicle puncture on embryo quality and pregnancy outcome. We also compared the clinical features and COH process with IVF during fresh cycle embryo transfer in clinical pregnancy (n = 774) and non-clinical pregnancy (n = 527) groups to explore the independent factors that influence pregnancy outcome.

Materials and methods

Ethical approval was obtained from the Ethics Committee of the Yantai Yuhuangding Hospital (No. 2015-169). Written informed consent was obtained from the patients before their inclusion in the study.

General information

From January 2016 to June 2018, *in vitro* fertilization-embryo transfer/intracytoplasmic sperm injection (IVF-ET/ICSI) assisted pregnancy treatment was given to patients (n = 2593) in the Department of Reproductive Medicine of Yantai Yuhuangding Hospital, using the luteal-phase GnRH-a long protocol (Diphereline, 0.05/0.03 mg by injection every day for 16 days, 0.1 mg/dose; Ipsen, Paris, France) to down-regulate the pituitary [estradiol (E2) <30 pg/mL; LH <3 mIU/mL]. Inclusion criteria were as follows: age ≤45 years, body mass index (BMI) 19 to 30 kg/m^2, basal follicle-stimulating hormone (FSH) <10 IU/L, normal uterine cavity as assessed through hysteroscopy, and normal maternal and paternal karyotypes. Exclusion criteria were as follows: a history of repeated implantation failure (RIF), severe uterine malformation, severe uterine adhesions, chromosomal abnormality, hydrosalpinx if

the fallopian tube had not been surgically removed or ligated, any contraindications to pregnancy, thyroid or adrenal dysfunction, neoplasia, severe impairment of renal or hepatic function, and use of medications that might interfere with study evaluations (e.g., hormonal medication, prostaglandin inhibitors, and psychotropic agents). Although the definition of RIF remains variable, many researchers consider failure of implantation after three or more embryo transfers or transfer of ten or more high-quality embryos to be the criterion.[3] Anovulatory patients were treated with compound oral contraceptive pill (OCP; Diane-35, Bayer, Leverkusen, Germany) beginning on day 5 of the menstrual cycle, and GnRH-a down-regulation therapy was initiated when 5 tablets of OCP remained. The initial dose of gonadotropin (Gn; Puregon, 75 IU/dose; Merck & Co. Inc., Kenilworth, NJ, USA) was determined according to the age of the patient, basal FSH level, number of antral follicles, BMI, ovarian volume, and history of previous ovarian surgery; the initial dose was usually 150 to 300 IU per day. During days 4 to 6 of treatment, the average diameter of each dominant follicle was measured by ultrasound.

When the ultrasound examination revealed 1 or 2 follicles with an average diameter ($\geq$4 mm) exceeding that of other follicles (and the number of other follicles was $\geq$4), these follicles were regarded as the dominant follicles and follicle puncture was performed. The patients were designated as the puncture group (n $=$ 48). After the procedure, the dose of Gn (50–75 IU) was increased to promote follicular development until the day of administration of human chorionic gonadotropin (HCG; 2000 IU/dose; Livzon Pharmaceutical Group Inc., Guangdong, China). When the diameter of the dominant follicles reached $\geq$14 mm, human menopausal gonadotropin (HMG, 75 IU/dose; Livzon

Pharmaceutical Group Inc.) was provided. When at least 2 dominant follicles had an average diameter $\geq$18 mm, an intramuscular injection of HCG (6000 IU) was given. After 34 to 36 hours, oocytes were retrieved and fertilized by conventional IVF or ICSI. After 72 hours, embryo transplantation was performed. After transplantation, vaginal progesterone soft capsules (100 mg/capsule; 200 mg every 8 hours daily; Capsugel, Colmar, France) were administered for corpus luteum support. Progesterone was administered continuously until 10 weeks of pregnancy or 14 days after ET if not pregnant.

In the control group (n $=$ 2545), B-mode ultrasound monitoring was performed during days 4 to 6 of the Gn treatment. Follicular development was relatively uniform, and there was no growth of dominant follicles (i.e., with average diameter exceeding that of other follicles by $\geq$4 mm); Gn was used continuously to promote follicular development until the day of HCG administration.

Puncture of large follicles

During days 4 to 6 of Gn treatment, the average diameter of 1 or 2 larger follicles exceeded that of other follicles by $\geq$4 mm. After routine disinfection of the vulva and vagina and under the guidance of vaginal B-mode ultrasound, a 17-gauge single-lumen ovum aspiration needle (Smiths Medical, Hythe, United Kingdom) was used to puncture the targeted larger follicles and aspirate the follicular fluid.

Oocyte retrieval, IVF, ICSI, and ET

A 17-gauge double-lumen ovum aspiration needle (Cook Medical, Brisbane, Australia) was used to retrieve oocytes under the guidance of vaginal B-mode ultrasound. After *in vitro* culture for 4 to 6 hours, IVF or ICSI was performed. After 16 to 20 hours,

fertilized oocytes were observed. Cleavage was observed at 48 hours after oocyte retrieval. Two high-quality embryos were selected for transplantation at 72 hours after oocyte retrieval.

Diagnosis of pregnancy

Blood β-HCG level was measured on day 14 after ET. Presence of a gestational sac was taken as an indicator of clinical pregnancy and observed on day 34 after ET.

Measurement of hormones

Venous blood samples were drawn during the second and third days of the menstrual cycle and used to measure basal levels of hormones, including E2, LH, FSH, testosterone (T), progesterone (P), pituitary prolactin (PRL), anti-Müllerian hormone (AMH), and inhibin B (INB). E2 and P were measured on the down-regulation day, oocyte retrieval day, and ET day; E2 and LH were measured on initiation day; and E2, LH, and P were measured on the HCG day.

Embryo rating and criteria for available embryos

In accordance with a published cleavage stage embryo scoring system[4] combined with work in our laboratory, we evaluated the quality of day 3 embryos. The grading criteria were as follows: Grade I, uniform cell size, regular shape, translucent, and fragmentation <5%; Grade II, the cells were slightly uneven, the shape was slightly irregular, the cytoplasm might have coarse particles, and fragmentation 6% to 20%; Grade III, uneven size and irregularly shaped cells, particles in cytoplasm, and fragmentation 21% to 50%; and Grade IV, very uneven cell size, many particles in cytoplasm, and fragmentation >50%. Day 3 embryos with 7 to 9 blastomeres and having morphology of Grade I or II or

fused embryos were considered high-quality embryos.

Parameters for laboratory observation

Parameters were calculated as follows: oocyte maturation rate (%) = number of mature oocytes/number of oocytes obtained × 100%; fertilization rate (%) = 2PN (2 pronuclear stage) fertilized oocytes/number of oocytes × 100%; cleavage rate (%) = 2PN cleavage embryo number/2PN fertilized oocyte number × 100%; high-quality embryo rate (%) = 2PN high-quality embryo number/2PN cleavage embryo number × 100%; clinical pregnancy rate (%) = clinical pregnancy number/number of transplant cycles × 100%.

Statistical methods

IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA) was used for analyzing data. Measurement data were expressed as means ± standard deviations (SD) or medians and quartiles (25% to 75%), and count data were expressed as frequencies. The independent samples t-test was used to compare normal and variance homogeneity, and the nonparametric rank sum test was used to compare data that were not normally distributed. Count data were tested mainly using the χ^2 test. Logistic stepwise regression analysis was used to analyze the factors influencing pregnancy outcome. A difference with a P-value < 0.05 was considered statistically significant.

Results

Age, E2 level on down-regulation day, initial dose of Gn, number of Gn days, total dose of Gn, and the total dose of HMG were significantly higher in patients of the puncture group (n = 48) than in those of the control group (n = 2545) ($P < 0.05$), whereas AMH, the number of follicles ≥16 mm on HCG day, and the volume of follicular

lavage fluid were significantly lower in patients of the puncture group than in those of the control group. We detected no significant differences in years of infertility, BMI, INB, basal endocrine markers, level of P on down-regulation day, levels of E2 and LH on initiation day, levels of E2, LH, and P on HCG day, endometrial thickness on HCG day, distance between droplet and uterine bottom during transplantation, or number of oocytes obtained between the two groups (Table 1).

The rate of secondary infertility was significantly higher in the puncture group than in the control group, whereas rates of high-quality embryos and implantation of embryos were significantly lower in the puncture group than in the control group ($P < 0.05$). However, there were no differences in OCP pretreatment rate, fresh cycle transplantation rate, oocyte maturation rate, 2PN fertilization rate, cleavage rate, or clinical pregnancy rate between the two groups (Table 2).

Table 1. Comparison of clinical features and measures of controlled ovarian hyperstimulation (COH) between the control group and the large follicle puncture group (means $\pm$ standard deviations).

Clinical characteristics	Control group	Puncture group	P-value
Number of cases	2545	48	
Age (year)	31.56 ± 0.07	33.29 ± 0.56	<0.01[*]
Infertility time (year)	3.78 ± 0.05	4.29 ± 0.53	0.16
AMH (ng/mL)	5.63 ± 0.086	3.68 ± 0.45	<0.01[*]
Inhibin B (ng/mL)	105.7 ± 3.14	105.1 ± 12.42	0.97
BMI (kg/m^2)	23.54 ± 0.07	23.79 ± 0.47	0.62
Basal E2 (pg/mL)	44.02 ± 1.7	48.80 ± 8.93	0.70
Basal LH (mIU/mL)	5.84 ± 0.07	5.26 ± 0.44	0.23
Basal FSH (mIU/mL)	6.72 ± 0.04	6.88 ± 0.26	0.55
Basal T (ng/mL)	0.29 ± 0.02	0.25 ± 0.02	0.68
Basal P (ng/mL)	0.94 ± 0.49	0.66 ± 0.12	0.43
Basal PRL (ng/mL)	18.54 ± 0.21	16.85 ± 1.31	0.28
E2 on down-regulation day (pg/mL)	170.1 ± 1.84	201.1 ± 14.41	0.02[*]
P on down-regulation day (ng/mL)	14.02 ± 0.15	14.43 ± 1.02	0.7
E2 on initiation day (pg/mL)	29.74 ± 3.9	23.18 ± 4.19	0.82
LH on initiation day (mIU/mL)	1.86 ± 0.018	1.98 ± 0.13	0.41
E2 on HCG day (pg/mL)	3591 ± 41.94	3137 ± 247.9	0.14
LH on HCG day (mIU/mL)	2.46 ± 0.023	2.20 ± 0.13	0.13
P on HCG day (ng/mL)	2.67 ± 1.72	1.04 ± 0.06	0.90
Initiation dose (IU)	200.3 ± 1.26	242.2 ± 7.6	<0.01[*]
Gn duration (day)	8.97 ± 0.036	9.63 ± 0.25	0.01[*]
Gn dose (IU)	1863 ± 12.13	2579 ± 110.0	<0.01[*]
HMG dose (IU)	149.0 ± 1.40	196.1 ± 47.92	<0.01[*]
Number of follicles $\geq$16 mm on HCG day	9.62 ± 0.09	8.17 ± 0.71	0.03[*]
Endometrial thickness on HCG day (mm)	11.49 ± 0.05	11.08 ± 0.34	0.25
Distance between droplet and uterine bottom (cm)	1.58 ± 0.05	1.51 ± 0.047	0.82
Number of oocytes obtained	10.21 ± 0.1	9.06 ± 0.76	0.12
Follicle lavage fluid (mL)	86.27 ± 0.87	69.89 ± 4.84	0.01[*]

[*]Values are significantly different between groups ($P < 0.05$)

AMH, anti-Müllerian hormone; BMI, body mass index; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; P, progesterone; PRL, prolactin; HCG, human chorionic gonadotropin; Gn, gonadotropins; HMG, human menstrual gonadotropin

Table 2. Comparison of clinical features and treatment outcomes between the control group and the large follicle puncture group.

Clinical factors	Control group	Puncture group	P-value
OCP therapy (%)	405/2545 (15.9)	6/48 (12.5)	0.51
Secondary infertility (%)	1174/2545 (46.1)	29/48 (60.4)	0.04[*]
Fresh cycle transplantation rate (%)	1573/2545 (61.8)	32/48 (66.7)	0.49
Oocyte maturation rate (%)	20,669/25,891 (79.8)	330/427 (77.3)	0.19
2PN fertilization rate (%)	18,393/25,891 (71.1)	286/427 (76)	0.07
Cleavage rate (%)	17,873/18,393 (97.2)	276/286 (96.5)	0.49
High-quality embryo rate (%)	11,469/17,873 (64.2)	161/276 (58.3)	0.04[*]
Implantation rate (%)	1186/2982 (39.8)	15/61 (24.6)	0.02[*]
Clinical pregnancy rate (%)	903/1573 (57.4)	16/32 (50)	0.40

*Values are significantly different between groups ($P < 0.05$)
OCP, compound oral contraceptive pill; 2PN = 2 pronuclear stage

Age ($P < 0.01$), infertility ($P = 0.04$), and BMI ($P < 0.01$) were lower in the clinical pregnancy group (n = 774) than in the non-clinical pregnancy group (n = 572). The levels of E2 and LH on HCG day ($P = 0.02$ and $P = 0.01$), number of follicles ≥ 16 mm on HCG day ($P = 0.04$), endometrial thickness on HCG day ($P < 0.01$), endometrial thickness on ET day ($P < 0.01$), number of oocytes obtained ($P < 0.01$), and number of transplanted embryos ($P < 0.01$) were higher in the clinical pregnancy group than in the non-clinical pregnancy group. The initial dose and total dose of Gn ($P < 0.01$ and $P = 0.03$) were lower in the clinical pregnancy group than in the non-clinical pregnancy group. The clinical pregnancy rate was lower with OCP treatment than without OCP treatment ($P < 0.01$) (Table 3).

In the multivariate logistic regression analysis, age, BMI, endometrial thickness on HCG day, number of oocytes obtained, number of transplanted embryos, and OCP treatment were the independent factors influencing pregnancy outcome. Considering all of the above factors, larger follicle puncture had no statistically significant effect on pregnancy outcome (Table 4).

Discussion

Since the birth of the first IVF-ET baby using natural cycle oocyte retrieval in 1978, the technology has developed rapidly over the past 40 years. In particular, improvements in the COH regimen have resulted in the simultaneous maturation of multiple follicles in one ovarian stimulation cycle. Simultaneous development of multiple follicles in one COH cycle increases the number of mature oocytes obtained, which compensates for the loss during oocyte retrieval, IVF, and ET, which increases the chance of transplanting high-quality embryos and increases the clinical pregnancy rate. However, although the COH protocol can be applied for different indications, the problem of unsynchronized maturation of follicles in COH has not yet been resolved.

The causes of unsynchronized maturation of follicles in COH

Causes of UFM in COH can be attributed to iatrogenic factors and patient factors. Iatrogenic factors include the choice of COH regimen and the initial Gn dose. The rate of UFM in COH is reported to be highest in the short-term regimen,

Table 3. Comparison of clinical characteristics and controlled ovarian hyperstimulation (COH) status in patients with different pregnancy outcomes (means ± standard deviations or medians with 25th and 75th quartiles in parentheses).

Clinical characteristics	Clinical pregnancy (774)	Non-clinical pregnancy (527)	$t/Z/\chi^2$	P-value
Age (year)	31.55 ± 3.26	32.4 ± 3.72	4.37	<0.01*
Infertility time (year)	3.65 ± 2.34	3.93 ± 2.61	2.01	0.04*
AMH (ng/mL)	3.78 (2.38, 6.01)	3.71 (2.17, 5.63)	−1.58	0.11
BMI (kg/m^2)	23.3 ± 3.52	23.91 ± 3.79	2.94	<0.01*
Basal E2 (pg/mL)	33.11 (24.54, 45.03)	34.34 (26.61, 45.79)	−1.18	0.24
Basal LH (mIU/mL)	4.88 (3.63, 6.35)	5.14 (3.8, 6.72)	−1.16	0.25
Basal FSH (mIU/mL)	6.72 (5.89, 7.81)	6.8 (5.75, 7.93)	−0.12	0.90
Basal T (ng/mL)	0.23 (0.16, 0.31)	0.23 (0.16, 0.34)	−0.70	0.48
Basal P (ng/mL)	0.57 (0.43, 0.75)	0.58 (0.43, 0.76)	−0.44	0.66
Basal PRL (ng/mL)	16.52 (12.51, 22.06)	16.46 (12.59, 22.29)	−0.20	0.84
E2 on initiation day (pg/mL)	8.99 (5, 15.83)	10.09 (5, 17.59)	−1.72	0.09
LH on initiation day (pg/mL)	1.75 (1.39, 2.24)	1.75 (1.37, 2.2)	−0.04	0.97
E2 on HCG day (pg/mL)	2724 (2006.5, 3295.75)	2560 (1791, 3242)	−2.30	0.02*
LH on HCG day (mIU/mL)	2.56 (1.88, 3.36)	2.41 (1.64, 3.23)	−2.51	0.01*
P on HCG day (ng/mL)	0.86 (0.66, 1.03)	0.84 (0.65, 1.07)	−0.14	0.89
E2 on ET day (pg/mL)	1620 (1214.5, 2145)	1587 (1160.5, 2104.5)	−1.01	0.31
P on ET day (ng/mL)	60 (60, 60)	60 (60, 60)	−1.80	0.07
Initiation dose (IU)	225 (175, 225)	225 (175, 250)	−3.04	<0.01*
Gn duration (day)	9 (8, 10)	9 (8, 10)	−0.75	0.45
Gn dose (IU)	1875 (1543.75, 2250)	1950 (1600, 2325)	−2.21	0.03*
HMG dose (IU)	150 (75, 150)	150 (75, 150)	−0.71	0.48
Number of follicles ≥16 mm on HCG day	8 (6, 10)	7 (5, 9)	−2.04	0.04*
Endometrial thickness on HCG day (mm)	12 (10, 13)	11 (10, 12)	−5.62	<0.01*
Endometrial thickness on ET day (mm)	1 (0.9, 1.2)	1 (0.8, 1.2)	−2.77	<0.01*
Number of oocytes obtained	8 (6, 10)	8 (5, 10)	−2.96	<0.01*
Number of transplanted embryos	2 (2, 2)	2 (2, 2)	−4.02	<0.01*
Follicle lavage fluid (mL)	80 (60, 100)	80 (60, 100)	−1.70	0.09
OCP treatment			7.04	<0.01*
No	677 (87.47)	433 (82.16)		
Yes	97 (12.53)	94 (17.84)		
Type of infertility			2.830	0.09
Primary infertility	401 (51.81)	248 (47.06)		
Secondary infertility	373 (48.19)	279 (52.94)		
Grouping			0.036	0.85
Control group	759 (98.06)	516 (97.91)		
Puncture group	15 (1.94)	11 (2.09)		

*Values are significantly different between groups ($P < 0.05$)
AMH, anti-Müllerian hormone; BMI, body mass index; E2, estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; P, progesterone; PRL, prolactin; HCG, human chorionic gonadotropin; ET, embryo transfer; Gn, gonadotropins; HMG, human menstrual gonadotropin; OCP, compound oral contraceptive pill

Table 4. Multivariate logistic regression results of the factors influencing pregnancy outcome.

Variable	B	SE	Wald	df	P-value	Odds ratio	95% CI for OR	
							Lower	Upper
Age	0.07	0.02	14.93	1	<0.01[*]	1.07	1.03	1.11
BMI	0.04	0.02	5.50	1	0.02[*]	1.04	1.01	1.08
Endometrial thickness on HCG day	−0.19	0.03	21.20	1	<0.01[*]	0.88	0.83	0.93
Number of oocytes obtained	−0.05	0.02	5.40	1	0.02[*]	0.96	0.92	0.99
Number of transplanted embryos	−0.87	0.23	13.84	1	<0.01[*]	0.42	0.27	0.66
OCP treatment vs. non-OCP treatment	0.38	0.17	4.85	1	0.03[*]	1.46	1.04	2.05
Puncture group vs. control group	−0.15	0.42	0.13	1	0.72	0.86	0.38	1.96
Constant	−0.01	0.86	0.00	1	0.99	0.99		

*Values are significantly different between groups ($P < 0.05$)
BMI, body mass index; HCG, human chorionic gonadotropin; OCP, compound oral contraceptive pill

followed by the ultra-long regimen, and lowest in the long-term regimen.[5] Cramer et al.[6] found that the short-term regimen did not inhibit physiological FSH in the luteal phase, and endogenous FSH gradually increased before Gn initiation. Some follicles with a lower FSH threshold begin to mature in the late luteal phase and administration of exogenous Gn may strengthen the maturation of unsynchronized follicles. Unsynchronized maturation of follicles reduces the pregnancy rate.[7] In the ultra-long protocol, GnRH-a can be uniformly released for 28 days. However, as the duration of Gn use increases in superovulation, the inhibitory effect of GnRH-a on the LH peak is gradually weakened, which affects follicular development.

Although the results of the current study suggest that follicular maturation is more synchronic with the long-term GnRH-a regimen, we identified 48 cases of UFM that underwent larger follicle puncture. With increasing age, the reproductive function of the ovary declines, the number of oocytes remaining in the ovary gradually decreases, and the remaining follicles are less sensitive to Gn. Thus, the same or higher dose of Gn cannot recruit sufficient follicles.[8] Macklon et al.[9] proposed that as levels of luteal-phase estrogen and progesterone decrease in older women, the negative feedback to the hypothalamus and pituitary might be weakened and the FSH level and FSH threshold increased, so that high doses of exogenous Gn are needed to recruit follicles. The initial dose of Gn increases with the patient's age. When the exogenous Gn level in older women is too low, some follicles that are less sensitive to FSH will not mature synchronously. In this study, we found that although the initial dose and total amount of Gn were significantly higher in the puncture group than those in the control group, the number of follicles ≥16 mm on HCG day was still lower in the puncture group. Whether the initial dose of Gn in the puncture group was still insufficient needs further research.

Patient factors that cause UFM include age and ovarian response. It has been reported that the rate of unsynchronized developing follicles increases with age; that is, the older the woman, the more likely it is that an early LH peak occurs.[5] Moreover, older women have an earlier increase in FSH level, leading to earlier follicular recruitment and advancement of follicular phase and ovulation, so that the number of antral and early antral follicles decreases, resulting in a decline of inhibin B, which

decreases and weakens the negative feedback suppression of FSH.[10] In the present study, we found that the average age of patients was significantly higher in the puncture group than in the control group, as was the E2 level on down-regulation day, suggesting that the antral follicle in this group of patients may have been recruited and matured before the use of GnRH-a in the luteal phase.

Factors improving the synchronization of follicular maturation

Steroid pretreatment with OCP, estrogen, and progesterone. The negative feedback effect of steroid hormones inhibits Gn secretion from the pituitary and improves the *in vivo* hormonal environment, but whether the OCP or E2 preconditioning regimen improves the synchrony of follicular development and IVF outcome remains controversial. Both OCP and synthetic progesterone have been used in clinical research of the COH cycle for nearly 20 years. Pretreatment with OCP has a clinical advantage in reducing the incidence of ovarian cysts, scheduling COH, and improving the synchronization of follicular maturation but it also increases the dose and duration of Gn treatments and increases the incidence of early pregnancy loss. It has been suggested that ceasing OCP 5 days before Gn initiation is optimal.[11–13] In this study, we found no correlation between follicular developmental dissonance and OCP pretreatment in patients before down-regulation. Because E2 secretion is the main factor inhibiting endogenous FSH during the transformation of luteal and follicle, E2 pretreatment has an inhibitory effect on FSH. Fanchin et al.[14,15] found that E2 pretreatment could improve follicular synchronization. In contrast to the GnRH antagonist (GnRH-ant) regimen without E2 pretreatment, E2 pretreatment increases the oocyte maturation rate, the number of available oocytes, the number of embryos, and the pregnancy rate. In addition, pretreatment of the luteal progesterone with dydrogesterone before Gn initiation can promote the uniformity of follicular maturation.[16]

GnRH-ant pretreatment. GnRH-ant has the effect of rapidly inhibiting the secretion of endogenous Gn. Therefore, premenstrual injection of GnRH-ant can prevent the increase of FSH in the luteal phase, prevent the advanced growth of the antral follicle, and promote the uniformity of follicular maturation. GnRH-ant simulates the down-regulation of GnRH-a. Moreover, GnRH-ant is short lived and dose dependent, thus avoiding the deficiencies of long-term treatment and reducing the menopause symptoms caused by the long-term GnRH-a regimen. Fanchin et al.[17] selected 25 volunteers for a study, in which each participant served as her own control, and found that injection of GnRH-a 4 days before menstruation inhibited the increase of FSH and the development of early follicles in the luteal phase, which, in turn, reduced variation of antral follicle diameter and improved the synchrony of follicular maturation. Saini et al.[18] found that GnRH-ant pretreatment in the first 1 to 5 days of Gn shortened the duration of Gn stimulation and increased synchronization of follicular development. No premature LH peak appeared and more high-quality oocytes and embryos were obtained, the pregnancy rate was reduced, and the rate of cycle cancellation caused by ovarian hyperstimulation syndrome was reduced.

Puncture of the large follicle. The E2 level of follicular fluid is significantly and positively correlated with oocyte nuclear maturation, fertilization, and embryo grading. A higher E2 level in follicular fluid results in a higher nuclear maturation rate.[19] In the ovulation induction cycle, because the

follicles respond differently to FSH, the follicles are not synchronized, and dominant follicles inhibit the growth of nondominant follicles. The faster-growing follicles may have nuclei and cytoplasm that are not synchronized, resulting in a rapid increase in follicular volume. The quality of the oocyte is not high and the oocyte maturation rate is reduced.[20,21] Puncture of larger follicles results in aspiration of steroid-rich follicular fluid, reduces circulating levels of steroid hormones, and eliminates endogenous LH peaks that may be induced, thereby eliminating the direct inhibition of adjacent follicles by dominant follicles, facilitating the synchronized development of the remaining follicles, and improving the oocyte maturation rate and pregnancy rate. Although the number of follicles ≥ 16 mm on HCG day and the rates of high-quality embryos and implantation of embryos were lower in the puncture group than in the control group, the clinical pregnancy rate was not significantly different between the two groups, suggesting that puncture of larger follicles can be used to increase synchronization of the remaining follicles. However, some studies have found that puncture of larger follicles increases the patient's pain and economic burden but does not improve treatment outcomes compared with patients in the non-puncture group. When unsynchronized development of follicles occurs in COH, the original regimen can be continued without the need for follicle puncture.[22] We found that after correcting for factors influencing pregnancy outcome such as age, BMI, endometrial thickness on HCG day, number of oocytes obtained, number of transplanted embryos, and OCP treatment, puncture did not significantly affect pregnancy outcome.

In summary, the age of the patient and level of AMH were found to be the main factors leading to unsynchronized maturation of follicles. After treatment with larger follicle puncture, the number of follicles ≥ 16 mm on HCG day was reduced and rates of high-quality embryos and implantation of embryos were reduced, although the clinical pregnancy rate was not affected. Further studies are needed to determine whether unsynchronized follicular development is caused by an insufficient initial dose of gonadotropins and whether large follicle puncture is needed.

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