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ASSISTED REPRODUCTION TECHNOLOGIES

Association between the proportion of dominant follicles and oocyte developmental competence

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Abstract
Purpose To explore the optimal timing for hCG triggering by investigating the impact of different proportion of dominant follicles on the oocyte developmental competence.
Methods One hundred ninety-eight infertile women were divided into three groups according to the proportion of dominant follicles on hCG day: (1) low: <15 % (n=66); (2) middle: 15–27 % (n=66); (3) high: >27 % (n=66). The grouping criteria were the bottom and top tertiles of the proportion of dominant follicles.
Results The gonadotropin dosage, duration and maximum follicle diameter in the low proportion group were lower than those in the middle and high proportion groups. Oocyte maturation and the abnormal fertilization rate in the low proportion group were lower than those in the middle and high proportion groups. The normal fertilization rate did not differ among the three groups. The cleavage rate and number of transferable embryos in the low proportion group were significantly higher than those in the high proportion group. The high-quality embryo rate, implantation rate, and pregnancy rate in the low proportion group were significantly higher than those in the middle and high proportion groups.
Conclusions A high proportion of dominant follicles are closely associated with impaired oocyte developmental competence and low pregnancy rate. These findings suggest that follicular overgrowth induced by delayed hCG triggering may undermine oocyte developmental competence and the proportion of dominant follicles may be a potential parameters for hCG triggering.

Keywords Ovarian stimulation · hCG triggering · Oocyte quality · Pregnancy outcome

Capsule Association between the proportion of dominant follicles and oocyte quality.

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Introduction

In vitro fertilization and embryo transfer (IVF-ET) has become an important therapeutic technique for infertility. Acquisition of good-quality oocytes through ovarian stimulation is a critical step for the success of IVF-ET. Human chorionic gonadotropin (hCG) administration is required for mimicking physiological luteinizing hormone (LH) surge and inducing oocytes to extrude the first polar body. Thus, the appropriate timing of hCG administration is extremely important during ovarian hyperstimulation.

Many studies have demonstrated that the oocyte quality retrieved from large follicles is often better than that from small follicles [1–3], and those oocytes from large follicles tend to develop high quality embryos. Therefore, some clinicians argue that the timing of hCG triggering should be delayed to prolong the duration of ovarian stimulation and stimulate enough follicular growth. Accordingly, it causes the inconsistency in the criteria for hCG triggering in different reproductive centers [4–6].

The proportion of dominant follicles is defined as the number of follicles with a diameter of 18 mm or larger divided by the total number of follicles in the ovary. The proportion of dominant follicles is not only determined by the number of dominant follicles, but also determined by the total number of follicles in the ovary. Theoretically, the proportion of dominant follicles is a good parameter in reflecting the global state of the follicles in the ovary. Besides that, the proportion of dominant follicles is mainly determined by the timing of hCG triggering. The latter hCG is injected, the higher proportion of dominant follicle will be. Therefore, the aim of this study was to explore the optimal timing for hCG triggering according to analyzing the impact of different proportion of dominant follicles on the oocyte developmental competence.

Materials and Methods

Subjects

This retrospective study was conducted at the center for reproductive medicine in Memorial Hospital of Sun Yat-sen University from October 2012 to March 2013. The study included 198 women who underwent intracytoplasmic sperm injection (ICSI) because their partners were diagnosed as having severe oligospermia and asthenospermia

The inclusion criteria were the patients' age ≤45 years, body mass index between 17–30 kg/m^2, and basal FSH level ≤20 IU/L. The interval time between two cycles is less than 1 year for the patients who underwent repeated cycles. The exclusion criteria included history of previous poor response, recurrent implantation failure (failure to achieve a pregnancy after three or more cycles), submucosal fibroids, intrauterine adhesion, congenital uterine malformation, hydrosalpinx, or ovarian endometriomas>3 cm diameter, low-dose-aspirin administration, HPV positive infection.

This study was approved by the Institutional Review Board of Sun Yat-sen University in March 2012 (NO.E2012003).

Ovarian stimulation protocol

All patients underwent a long and step up protocol for ovarian stimulation. A gonadotropin-releasing hormone agonist, diphereline (Ipsen Pharma Biotech, France) was injected subcutaneously, 1.25 mg once for the downregulation of pituitary function. After 2 weeks, gonadotropin (150-300 IU, Gonal-F, Merck Serono, Germany) was injected subcutaneously for ovarian hyperstimulation. On the average, ultrasound monitoring was conducted five or six times during ovarian stimulation program, depending on the follicular growing speed. A dose of 10,000 IU hCG (Northern Pharma Biotech, China) was injected intramuscularly when at least two dominant follicles reached the diameter of 18 mm or larger. The proportion of dominant follicles is defined as the number of follicles with a diameter of 18 mm or larger divided by the total number of follicles in the ovary. All the follicles which were legible under ultrasound detection were included for counting the total number of follicles. The proportion of dominant follicles was sorted in ascending order and divided by tertiles (i.e. the bottom 1/3 and top 1/3 of the proportion of dominant follicles based on the previous hyperstimulation record). As a result, the bottom and top 1/3 boundaries of the proportion of dominant follicles were 15 and 27 %, respectively. Therefore, the patients were divided into three groups according to the proportion of dominant follicles on hCG triggering day: (1) low: <15 % (n=66); (2) middle: 15–27 % (n=66); (3) high: >27 % (n=66). At 36 h after hCG injection, cumulus-oocyte complexes (COCs) were collected with a 17-gauge needle under ultrasound guidance.

Oocyte and Embryo Assessment

A total of 2426 COCs were collected in this study. The grading criteria for COCs were as follows: grade I, compact cumulus and translucent ooplasm (n=808); grade II, less compact cumulus and dark ooplasm (n=898); and grade III, dark and expanded cumulus and dark ooplasm (n=720). Oocyte maturation was examined under an inverted micoscope. If the first polar body was extruded, the oocyte was regarded as being at the MII stage. An oocyte at the MII stage was fertilized via the ICSI procedure. The oocyte maturation rate refers to the number of MII oocytes/total number of all retrieved oocytes. Oocyte fertilization was observed 18–19 h after ICSI. Normal fertilization was confirmed when two pronuclei were observed in the cytoplasm. The normal fertilization rate refers to the number of fertilized oocytes/total number of all retrieved oocytes. Abnormal fertilization was confirmed when one or more than two pronuclei were found in the cytoplasm. The abnormal fertilization rate refers to the number of abnormal fertilizated oocytes/total number of all retrieved oocytes. Embryo cleavage was examined 43–45 h after ICSI. Normal embryo cleavage was defined when the fertilized egg developed into an embryo with 4–6 blastomeres. The cleavage rate refers to the number of cleavaged zygotes/total number of all zygotes. Embryo evaluation was conducted 67–69 h after ooctye fertilization. Transferable embryos had to have at least four blastomeres with a uniform size and less than 20 % of the fragment proportion, whereas high-quality embryos had to have 7–9 blastomeres with a uniform size and less than 10 % of the fragment proportion. The high-quality embryo rate refers to the number of high-quality embryo/total number of all embryos. No more than three embryos were transferred into the uterus on day 3. The implantation rate refers to the number of gestational sacs/total number of transferred embryos. Clinical pregnacy was diagnosed when both the gestational sac and fetal heart beat were observed 5 weeks after embryo transfer by ultrasound.

Statistical analysis

One-Sample Kolmogorov-Smirnov test was used to examine the normal distribution of all data. Some data were transformed into a normal distribution by a logarithmic method. The variance analysis (least significant difference, LSD) and chi-square analysis were used to compare the data among different groups. Data analysis was conducted using SPSS 11.5 (SPSS, Inc., Chicago, IL, USA), and $P<0.05$ was considered statistically significant.

Results

Comparison of clinical parameters after ovarian stimulation

Among the three groups, there were no significant differences in the following paraemters: age, body mass index (BMI), ovarian volume, basal antral follicle count, average number of previous cycles and basal sex hormone levels (Table 1). The mean proportions of dominant follicles in the low, middle, and high proportion groups were 8.73 %, 20.10 %, and 43.31 %, respectively. The gonadotropin dosage in the low proportion group (2113.40±906.29 IU) was significantly lower than that in the middle proportion group (2483.68±1021.78 IU), and the latter was significantly lower than that in the high proportion group (2640.93±1179.33 IU). The gonadotropin duration in the low proportion group (10.06±2.94 days) was significantly lower than that in the middle proportion group (12.76±3.12 days), and the latter was significantly lower than that in the high proportion group (13.21±4.77 days). The average number of dominant follicles in the low proportion group (2.35±1.45) was significantly lower than that in the middle proportion group (4.03±2.07), and the latter was significantly lower than that in the high proportion group (5.79±1.92). Moreover, the maximum diameter of dominant follicles in the low proportion group (20.41±2.04 mm) was significantly smaller than that in the middle proportion group (21.6±1.99 mm), which was significantly lower than that in the high proportion group (22.07±2.31 mm). However, the ratio of estradiol to dominant follicle number in the low proportion group (537.16±108.07) was significantly higher than those in the middle (261.67±96.32) and high (211.85±85.53) proportion groups. There was no significant difference in the average number of retrieved ooctyes among the three groups (Table 2).

Comparison of oocyte and embryo developmental parameters

The ratio of grade I COCs in the low proportion group (26.67 %) was significantly higher than those in the middle (17.32 %) and high (15.15 %) proportion groups, and the ratios of grade II and III COCs in the low proportion group (73.18 % and 0.16 %, respectively) were significantly lower than those in the middle (80.44 % and 2.23 %,, respectively) and high (79.22 and 5.63 %, respectively) proportion groups. The oocyte maturation rate in the low proportion group (80.60 %) was significantly lower than those in the middle (84.38 %) and high (91.49 %) proportion groups, but there was no significant difference between the rates of the middle and high proportion groups.

There was no significant difference in the normal fertilization rate among the three groups. The abnormal fertilization rate and cleavage rate in the low proportion group (6.51 and 89.80 %, respectively) were not significantly different from those of the middle proportion group (6.62 and 89.23 %, respectively), but both rates in the low and middle proportion groups were significantly lower (abnormal fertilization rate) or higher (cleavage rate) than those in the high porortion group (12.13 % and 78.08 %, respectively). The numbers of tranferable embryos in the low (6.46±2.82) and middle (5.50±4.18) proportion groups were significantly higher than

Table 1 General information for the subjects

Parameter	Low proportion	Middle proportion	High proportion	*P* value
Cases, n	66	66	66	–
Age (years)	32.63±4.46	32.57±4.95	32.45±6.67	0.24
Age range (years)	27~38	27~38	25~39	–
BMI (kg/m^2)	21.66±3.42	21.15±2.91	21.26±3.08	0.33
Ovarian volume (cm^3)	5.80±3.28	5.68±2.72	5.28±2.69	0.82
Antral follicle count	6.53±3.41	6.25±2.99	6.28±3.53	0.18
Average number of	0.62±0.12	0.58±0.09	0.65±0.13	0.82
FSH (IU/L)	8.70±3.44	8.27±2.82	8.63±2.13	0.53
LH (IU/L)	5.15±3.29	5.28±3.34	4.97±3.19	0.79
Estradiol (pg/mL)	14.97±4.38	18.56±7.18	16.11±4.56	0.26
Testosterone (ng/L)	1.69±0.83	1.92±0.62	1.47±0.64	0.73
Prolactin (pg/mL)	16.59±8.33	16.33±8.90	16.11±9.16	0.87

The variance analysis and chi-square analysis were used for data comparisons. There were no significant differences in the general parameters among the three groups

Table 2 Clinical parameters for ovarian stimulation

Parameter	Low proportion	Middle proportion	High proportion	*P* value
Proportion of dominant follicles*	8.73 %	20.10 %	43.31 %	<0.001
Gonadotropin dosage (IU)*	2113.40±906.29	2483.68±1021.78	2640.93±1179.33	<0.001
Gonadotropin duration (days)*	10.06±2.94	12.76±3.12	13.21±4.77	<0.001
Number of total follicles	13.56±5.26	13.82±5.28	13.17±6.31	0.56
Number of dominant follicles*	2.35±1.45	4.03±2.07	5.79±1.92	<0.001
Maximum follicle diameter	20.41±2.04	21.61±1.99	22.07±2.31	<0.001
Estradiol /dominant follicle	537.16±108.07	261.67±96.32	211.85±85.53	<0.001
Average number of oocytes retrieved	10.03±2.29	10.88±2.61	9.07±2.98	0.31

The variance analysis and chi-square analysis were used for data comparisons. * $P<0.05$, there were significant differences among the three groups

that in the high porortion group (4.34±4.21). The number of high-quality embryos in the low proportion group (60.19 %) was significantly higher than those in the middle (46.67 %) and high (41.93 %) proportion groups. Moreover, the implantation rate in the low proportion group (41.41 %) was significantly higher than those in the middle (31.58 %) and high (29.27 %) proportion groups, and accordingly, the pregnancy rate in the low proportion group (56.60 %) was significantly higher than those in the middle (48.33 %) and high (40.74 %) proportion groups (Table 3).

Discussion

HCG timing is a critical factor for oocyte maturation and subsequent development during ovarian simulation. In some reproductive center, hCG is delayed administrated for promoting the growth of follilces, which is though to be benefical for improving oocyte quality [7–9]. In the present study, we analyzed the correlation between the proportion of dominant follicles and oocyte developmental competence, aiming to explore the optimal timing for hCG triggering.

In our center, the basic criterion for hCG triggering was at least two follicles of 18 mm or larger. However, it was only the lowest standard for hCG triggering. We often delay the timing for hCG triggering and prolong the ovarian stimulation days in the practical works, which aimed to simulate enough follicular growth and harvest as many mature eggs as possible. As a result, much more than two dominant follicles were formed in the high proportion group and the largest diameter of dominant follicles was even larger than 22 mm. The proportion and diameter of dominant follicles were positively associated with the duration of ovarian stimulation.

According to the proportion of dominant follicles, all patients were divided into low, middle, and high proportion groups. We observed significant differences in oocyte development, embyro quality, and final pregnancy outcome among the three groups. Consistent with previous findings [10, 11], the abnormal fertilization rate in the high proportion group was higher than those in the low and middle proportion groups. Compare with the middle and high proportion groups, the maximum diameter of dominant follicles was smaller in the low proportion group, but the cleavage rate, high-quality embryo rate,

Table 3 Oocyte and embryo developmental parameters

Parameter	Low proportion	Middle proportion	High proportion	*P* value
Total number of COCs	808	898	720	–
Grades of COCs				
Oocyte maturation rate*	80.60 %	84.38 %	91.49 %	<0.001
Normal fertilization rate	70.05 %	70.58 %	72.56 %	0.89
Abnormal fertilization rate*	6.51 %	6.62 %	12.13 %	0.036
Cleavage rate*	89.80 %	89.23 %	78.08 %	0.028
Number of transferable embryos*	6.46±2.82	5.50±1.18	4.34±1.21	0.027
High-quality embryo rate*	60.19 %	46.67 %	41.93 %	0.039
Implantation rate*	41.41 %	31.58 %	29.27 %	0.042
Pregnancy rate*	56.60 %	48.33 %	40.74 %	0.036

The variance analysis and chi-square analysis were used for data comparisons. * $P<0.05$, there were significant differences among the three groups implantation rate, and pregnancy rate were significantly higher. These data indicate that the overgrowth of dominant follicles may have a negative impact on oocyte quality. The grade system for COCs to some extent can reflect the maturation state [12–14]. As the proportion of dominant follicles increased, the ratio of mature COCs (grade II) increased, and the ratio of aging COCs (grade III) also increased. Additionally, the steroids secreted by the granulosa cells may also reflect the oocyte quality [15–17]. Interestingly, when we compared the ratio of estradiol to dominant follicles on the day of hCG triggering, the ratio of estradiol to dominant follicles decreased significantly as the proportion of dominant follicles increased. The average estradiol level per dominant follicle was nearly 537 pg/mL in the low proportion group, whereas the level decreased to about 211 pg/mL in the high proportion group, which may reflect impaired follicular function. In accord with previous studies [18–20], these findings indicate that the high proportion caused by delaying the timing for hCG triggering may lead to oocyte post-maturity and unsatisfied pregnant outcome.

Tradditionally, the number of dominant follicle was often used for determing the timing for hCG triggering. In some cases, the patients may have asynchronous folliculogenesis during ovarian hypersimulation [21, 22], and then the developmental state of a few dominant follicles cannot represent the global state of all follicles in the ovaries. Therefore, it is not appropriate for all patients to decide the timing of hCG injection based only on the number of dominant follicles. For the first time, the proportion of dominant follicle was confirmed to be closely associated with oocyte developmental compentence in the present study. The present study suggests that the proportion of dominant follicle is closely associated with oocyte quality and may be used as a potential guiding parameter for hCG triggering. However, the current study is a retrospective one, which may limit the application of the results. In the future, the prospective study is still needed to verify the conclusion.

Taken together, the present study investigates the impact of different proportion of dominant follicles on the oocyte developmental competence. The results indicate that a high proportion of dominant follicles is closely associated with imparied oocyte developmental competence and low pregnancy rate, which may provide evidences for determining a suitable timing for hCG triggering. These findings suggest that the follicular overgrowth during ovarian stimulation may lead to the post-maturity of oocyte and finally caused unsatisfied pregnant outcome. Besides that, the proportion of dominant follicles may be used as a potential parameter for hCG triggering. In the future, A prospective, randomized and controlled study is still needed for verify the conclusion.

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