A study of follicular development and oocyte maturity predicted by transvaginal ultrasound on the day of human chorionic gonadotropin injection

Xia Chen, Xiao-Wen Liang, Jing-Hui Fang, Zhi-Yi Chen

Department of Ultrasound Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510150, China.

Quality of oocytes is closely related to the pregnancy outcome of in vitro fertilization (IVF). During the process of oocyte maturity, human chorionic gonadotropin (hCG) can simulate the effects of luteinizing hormone peak, which can further accelerate the maturation of follicles. The right time of hCG injection is a decisive factor for retrieving high-quality oocytes.[1] The optimal hCG injection time is usually determined using average diameter of follicle measured by transvaginal ultrasound. However, the threshold value of the diameter is still controversial.[2] The diameter measurement becomes increasingly less reliable when there are numerous follicles of different sizes and irregularly shaped structures during controlled ovarian hyperstimulation (COH) cycle. Follicle angiogenesis has been proved to be essential for the oocytes maturity in recent years.[3] But there are still few quantitative analyses of multi-ultrasound parameters of peri-follicular blood flow (PFBF) and the oocyte quality. This study mainly focuses on the morphology (like threshold of follicle diameter) and PFBF indicators for follicles on the hCG injection day during IVF cycle. The relationships between these indicators and the follicular development and oocyte maturity were systematically analyzed.

Thirty-two infertile women undergoing IVF-embryo transfer (ET) in the Reproductive Centre at the Third Affiliated Hospital of Guangzhou Medical University from June 2018 to October 2018 were enrolled prospectively. A total of 211 follicles were collected randomly from these patients. According to the average diameter on the day of hCG injection, these follicles were divided into four groups: Group A contained small follicles with the average diameter of ≥ 12 mm and < 15 mm (potential mature follicles); Group B contained medium-sized follicles with the average diameter of ≥ 15 mm and < 18 mm (premature follicles); Group C contained large follicles with the average diameter of ≥ 18 mm and < 23 mm (mature follicles); and Group D contained ultra-large follicles with the average diameter ≥ 23 mm (postmature follicles). Each patient had signed an informed consent for obtaining and analyzing their clinical data prior to the initiation of IVF-ET treatment. The study was approved by the Third Affiliated Hospital of Guangzhou Medical University Medical Ethics Review Board (No. 2018-73).

All ultrasonographic examination was performed by a physician who had 10 years of experience using Philips IU22 (Royal Philips, Eindhoven, Netherlands) on the day of hCG injection. Parameters of each follicle with a diameter ≥ 12 mm were measured and recorded. PFBF related parameters like peak systolic velocity (PSV), resistance index (RI) and grading system were measured. The grading system of PFBF used the semi-quantitative method proposed by Bhal et al.[4] The follicles were divided into four grades based on the percentage of blood flow accounting for follicle circumference with color Doppler blood flow signal of PFBF: Grade I indicated a percentage of < 25%, grade II indicated a percentage of 25% to 49%, grade III indicated a percentage of 50% to 75%, and grade IV indicated a percentage > 75%. Grades I, II, III, and IV were assigned scores of 1, 2, 3, and 4, respectively, while no blood flow was assigned a score of 0.

During the process of oocyte retrieval, oocyte-corona-cumulus complexes (OCCCs) were identified, and oocytes maturity was assessed. Subsequently, single-embryo culture was performed, and the fertilization and cleavage of each oocyte were documented. The oocytes maturity was divided into 3 stages: metaphase II (MII), metaphase I (MI) and germinal vesicle (GV) stage. The maturity of each
oocytes was scored by assigning grades of 2, 1, and 0 to oocytes of stages MII, MI, and GV, respectively; degenerated oocytes received a grade of 0. The number of immature oocytes (in the MI and GV stages) and the number of mature oocytes (in the MII stage) were recorded. The oocyte maturation rate was calculated as a percentage of the number of MII stage oocytes in the total number of oocytes. In addition, the oocytes were classified according to the number of pronuclei present. The categories included oocytes with no pronucleus (0PN), 1 pronucleus (1PN), or 2 pronuclei (2PN) and those with a polynucleus (PPN). Oocytes displaying 0PN, 1PN, 2PN, and PPN were scored 0, 1, 2, and 1, respectively. In this study, 2PN was employed as a marker of normal fertilization, and 0PN, 1PN, and PPN indicated aberrant fertilization. Fertilization rate was calculated through this formula: normal fertilization rate = (2PN/the total number of oocytes) × 100%.

Cleavage and embryo quality were graded using a scoring scale based on embryonic developmental morphology, the number and morphology of blastomeres, and the proportion of cytoplasmic debris in the embryo. The grading criteria were as follows: Grade I, debris proportion ≤5%; Grade II, debris proportion 6–20%; Grade III, debris proportion 21–50%; Grade IV, debris proportion >50%. Cleavage of grades I, II, III, and IV was scored 4, 3, 2, and 1, respectively; lack of cleavage was scored 0. After scoring according to the aforementioned criteria, high-quality embryos were defined as grades I and II on the third day. Each instance of a high-quality embryo was scored 1, and each instance of a non-high-quality embryo was scored 0. The cleavage rate and the proportion of high-quality embryos were calculated as follows: cleavage rate = (number of cleaved embryos/number of fertilized embryos) × 100%; the proportion of high-quality embryos = (number of high-quality embryos/total number of embryos) × 100%.

All data were analyzed and processed using IBM SPSS Statistics 22.0 software package (Armonk, NY, USA). The measurement data with non-normal distribution were presented as median (Q1, Q3). Kruskal-Wallis test was used for comparison between multiple groups and a Dunn-Bonferroni test for post hoc comparisons. Chi-square test was used to compare rates between different groups. Spearman coefficient was used to analyze the correlation between parameters and the oocyte maturity. P < 0.05 was considered statistically significant.

Our research showed that with the increase of follicle average diameter, peak systolic velocity of follicle (PSVF) of PFBF was gradually increased while RI was decreased. Apart from group C vs. group D and group B vs. group D, there were significant differences among different groups in PFBF score (A vs. B: 0.53 [0.1, 0.96] vs. 1.86 [1.34, 2.38], P < 0.001; A vs. C: 0.53 [0.1, 0.96] vs. 2.47 [1.82, 3.12], P < 0.001; A vs. D: 0.53 [0.1, 0.96] vs. 2.51 [1.66, 3.36], P = 0.001; B vs. C: 1.86 [1.34, 2.38] vs. 2.47 [1.82, 3.12], P < 0.001). From group A to group D, the maturation rate (A: 59.6%, B: 86.1%, C: 97.1%, D: 100%), normal fertilization rate (A: 36.2%, B: 84.8%, C: 91.3%, D: 80.0%) and cleavage rate (A: 81.1%, B: 97.4%, C: 98.5%, D: 100%) were gradually increased, indicating that the increase of follicle average diameter on the day of hCG injection resulted in a good oocyte maturity and an increase in the number of fertilization and cleavage. However, compared with group C, the normal fertilization rate in group D was decreased significantly (80.0% vs. 91.3%, χ² = 57.167, P = 0.007). The percentage of high-quality embryos was also decreased when the follicle average diameter was ≥23 mm (C vs. D: 89.6% vs. 70.0%, χ² = 12.550, P = 0.022).

Spearman correlation analysis showed the relationship between the follicle average diameter and oocyte maturity. The follicle average diameter showed significant correlations with the PPBF grade (r = 0.680, P = 0.001), PSVF (r = 0.709, P = 0.010), oocyte maturation score (r = 0.394, P = 0.001), cleavage score (r = 0.523, P = 0.003) and the number of high-quality embryos (r = 0.411, P = 0.008). RI of PFBF was negatively correlated with the follicle average diameter with the correlation coefficient of −0.723 (P = 0.003).

The relationships among individual parameters of follicular blood flow on the day of hCG injection and subsequent laboratory indicators were analyzed. PFBF grade exhibited significant correlation with oocyte maturation score, fertilization score, cleavage score, and the number of high-quality embryos, as shown by correlation coefficients of 0.483 (P = 0.003), 0.629 (P = 0.004), 0.630 (P = 0.042), and 0.367 (P = 0.008), respectively. PSVF of PFBF showed significant correlation with oocyte maturation score, fertilization score, cleavage score, and the number of high-quality embryos, as evidenced by correlation coefficients of 0.346 (P = 0.007), 0.529 (P = 0.021), and 0.419 (P = 0.008), respectively. RI of PFBF showed significant correlation with

![Figure 1: ROC curves of follicle average diameter, PFBF grade and PSVF in predicting oocyte maturity. PFBF: Peri-follicular blood flow; PSVF: Peak systolic velocity of follicle; ROC: Receiver operating characteristic.](image-url)
Multi-ultrasound parameters (including morphology and blood flow indicators) measured on the day of hCG injection provide a comprehensive approach that can be used to assess the quality and maturity of follicles. In the future, a larger sample size, grouping by age, and three-dimensional power Doppler technique can be employed to investigate quantitative parameters that allow accurate and comprehensive assessment of ovarian reserve function and ovarian responsiveness.

Funding
This work was supported by the grants from the National Natural Science Foundation of China (No. 81971621, No. 81671707), the Natural Science Foundation of Guangdong Province (No. 2019A151501212), Major Research Projects of Universities in Guangdong Province (No. 2019KZDZX1032), the Research Fund for Lin He’s Academician Workstation of New Medicine and Clinical Translation, Scientific and Technological Livelihood Projects of Liwan District (No. 201904003), and the Youth Foundation of Scientific Research of the Third Affiliated Hospital of Guangzhou Medical University (No. 2018Q18).

Conflicts of interest
None.

References
1. Cohen B, Bijkerk A, Van der Poel S, Ombelet W. IUI: review and systematic assessment of the evidence that supports global recommendations. Hum Reprod Update 2018;24:300–319. doi: 10.1093/humupd/dmx041.
2. Wirleitner B, Okhowat J, Vistejnová L, Kráľčíková M, Karliková M, Vanderverzalmen P, et al. Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval. Ultrasound Obstet Gynecol 2018;51:118–123. doi: 10.1002/uog.18955.
3. Hafner D, Zivkovic SV, Bauman R, Šiláková J, Papič N, Lepej SZ. Follicular fluid vascular endothelial growth factor is associated with type of infertility and interferon alpha correlates with endometrial thickness in natural cycle in vitro fertilization. Reprod Biol 2018;18:289–294. doi: 10.1016/j.repbio.2018.06.001.
4. Bhal PS, Pugh ND, Gregory L, O’Brien S, Shaw RW. Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: a study using transvaginal power Doppler. Hum Reprod 2001;16:1682–1689. doi: 10.1093/humrep/16.8.1682.
5. Revelli A, Martiny G, Delle Piano L, Benedetto C, Rinaudo P, Tur-Kaspa I. A critical review of b-dimensional and three-dimensional ultrasound techniques to monitor follicle growth: do they help improving IVF outcome. Reprod Biol Endocrinol 2014;12:107. doi: 10.1186/1477-7827-12-107.
6. Venets CA, Kolibianakis EM, Boudou JK, Lainas GT, Sfostouris IA, Tarlatzis BC, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. Hum Reprod 2015;30:684–691. doi: 10.1093/humrep/deu362.

How to cite this article: Chen X, Liang XW, Fang JH, Chen ZY. A study of follicular development and oocyte maturity predicted by transvaginal ultrasound on the day of human chorionic gonadotropin injection. Chin Med J 2021;134:731–733. doi: 10.1097/CM9.0000000000001341.