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

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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. 2018-75).

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

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oocyte 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 (Q$_1$, Q$_3$). 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 (PSV$_F$) 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, 1.06] *vs.* 1.86 [1.34, 2.38], $P < 0.001$; A *vs.* C: 0.53 [0, 1.06] *vs.* 2.47 [1.82, 3.12], $P < 0.001$; A *vs.* D: 0.53 [0, 1.06] *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%, $\chi^2 = 57.167$, $P = 0.007$). 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%, $\chi^2 = 12.550$, $P = 0.022$).

Spearman correlation analysis showed the relationship between the follicle average diameter and oocyte maturity. The follicle average diameter showed significant correlations with the PFBF grade ($r = 0.680$, $P = 0.001$), PSV$_F$ ($r = 0.709$, $P = 0.010$), 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 ($P = 0.005$).

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 0.485 ($P = 0.003$), 0.629 ($P = 0.004$), 0.650 ($P = 0.042$), and 0.567 ($P = 0.008$), respectively. PSV$_F$ 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 ($P = 0.007$), 0.405 ($P = 0.003$), 0.529 ($P = 0.021$), and 0.419 ($P = 0.008$), respectively. RI of PFBF showed significant correlation with
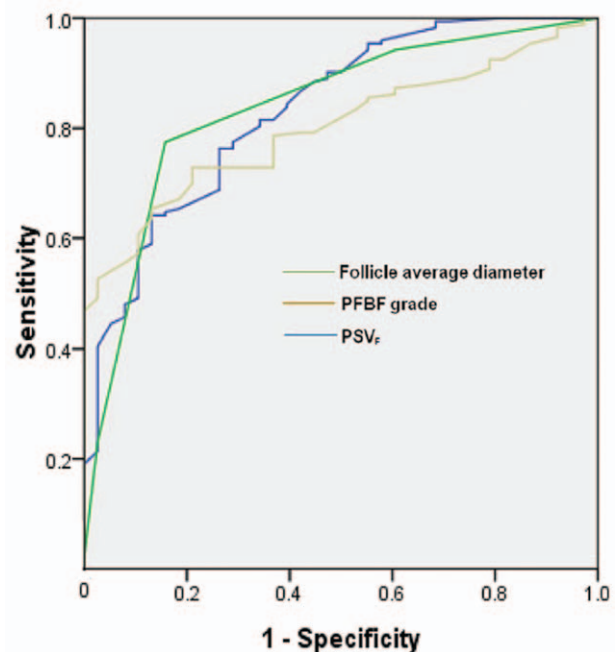
Figure 1: ROC curves of follicle average diameter, PFBF grade and PSV$_F$ in predicting oocyte maturity. PFBF: Peri-follicular blood flow; PSV$_F$: Peak systolic velocity of follicle; ROC: Receiver operating characteristic.

oocyte maturation score, fertilization score, cleavage score, and the number of high-quality embryos, as evidenced by correlation coefficients of −0.319 ($P = 0.018$), −0.371 ($P = 0.008$), −0.723 ($P = 0.001$), −0.480 ($P = 0.004$), and −0.424 ($P = 0.031$), respectively.

In the prediction of oocyte maturity, areas under the receiver operating characteristic (ROC) curve (AUCs) for follicle average diameter, PFBF grade and PSV_F were 0.832, 0.837, and 0.800, respectively. It indicated that these three parameters might have good diagnostic value in assisted reproductive therapy. The diagnostic cutoff values of these parameters were 15.65 mm, 1.5 (that is 37.5% of follicle circumference), and 8.45 cm/s, respectively [Figure 1].

It is a delicate task to determine the optimal timing of hCG injection for promoting final oocyte maturation in clinical practice. Administration of the hormone too early, at a time when the follicles remain morphologically and functionally immature, may cause granulosa cells to respond inappropriately to the hormone.[5] Clinical assessment of oocyte quality is mainly based on the follicle diameter, its morphology, and the morphology of OCCCs. In recent studies, transvaginal ultrasound was proved to be useful for the direct observation of follicular development and blood perfusion of the area surrounding the follicle. According to our study, we found that an increase in the follicle average diameter was associated with an elevation in intraovarian blood flow score and PSV as well as with a reduction in RI. In other words, large follicle sizes on the day of hCG injection indicate rich surrounding vascularization, high blood flow velocity, and a reduced resistivity index. However, once the follicular diameter reached 23 mm, there was no apparent increase in the PFBF grade or in the PSV. Furthermore, on the day of hCG injection, the PFBF grade displayed intermediate levels of positive associations with oocyte maturity, fertilization score, cleavage score, and high-quality embryo number. PSV_F exhibited significant positive associations with oocyte maturity, fertilization score, cleavage score, and high-quality embryo number, while RI showed significant negative associations. High-quality follicles are characterized by a specific pattern in which the PSV of the perifollicular blood flow increases and the RI decreases as the follicles develop. An increased peri-follicular blood flow signal implies neovascularization of the follicular wall or of the region around the follicles. This is one of the necessary conditions for follicular wall rupture and subsequent ovulation. Our data indicated that when multiple follicles with PFBF greater than 37.5% are present or when the PSV_F of multiple follicles measured in the vicinity of the follicular wall is greater than 8.45 cm/s, it can be assumed that most follicles are mature. The mechanism of low fertilization rate and low high-quality embryo rate of large follicles (≥ 23 mm) may be attributed to the degeneration of oocytes. When a follicle of optimal size continues to grow, the oocyte begins to degenerate which often manifests abnormal fertilization.[6]

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.

Conflicts of interest

None.

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