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Live Birth Rate of Assisted Reproductive Technologyin Sonography-based Automated Volume Calculation (SonoAVC)

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Abstract

Objective: To investigate the live birth rate of assisted reproductive technology in Sonography-based automated volume calculation (SonoAVC) and relative influencing factors.

Methods: 58 cases of infertile women underwent in vitro fertilization and embryo transfer (IVF-ET) in the reproductive medicine center of our hospital were retrospectively analyzed. Long term ovarian stimulation were performed using GnRH antagonist, oocyte maturation and blastocyst development were traced since the implement of ovarian stimulation, with 26 cases underwent conventional 2D ultrasonographyassigned to 2D group and 32 cases underwent SonoAVC to 3D group, two kinds of ultrasound techniques were compared to evaluate the ovarian volume and number of follicles. The effect of 3DSonoAVC on the periodic treatment of IVF using GnRH antagonist was evaluated in terms of clinical pregnancy rate and live birth rate. Oocyte maturity, OCCC score, fertilization, and cleavage rate were assessed among follicles of different diameters. Univariate and multivariate analysis of pregnancy outcomes in assisted reproductive technology affecting 3DSonoAVC were performed using Cox regressive model.

Results: Both 2D and 3D ultrasound techniques exerted no effect on pregnancy outcomes (P>0.05). Compared with 2D ultrasound technology, however, ovarian volume and number of follicles showed higher level in the detection of 3DSonoAVC technology with shorter counting time (P<0.05) and the proportion, follicle OCCC score, fertilization rate and cleavage rate of the muture oocyte with follicle diameter ≧16mm significantly increased (P<0.05). The factors affecting the pregnancy outcomes of 3D group were analyzed by Cox regressive model, in which the results of single factor analysis showed that age, infertility, endometrial thickness and quality of transplanted embryos were correlated with the pregnancy outcome of assisted reproductive technology in SonoAVC(P<0.05) , and the multi-factor analysis (both P<0.05) showed that infertile patients with transplanted embryo of excellent quality and 8 mm ≤ endometrial thickness ≤ 16 have a good pregnancy outcome in the detection of assisted reproductive technology in SonoAVC.

Conclusion: Assisted reproductive technology in SonoAVC showed no better pregnancy outcomes than conventional 2D ultrasonography. Age, embryo quality and endometrial thickness of HCG day were independent factors influencing clinical pregnancy outcome. The clinical pregnancy rate of patients with age ≥35 significantly reduced. Endometrial thickness of HCG day ranged from 8 to 16 mm could improve clinical pregnancy rate.

Keywords: 3DSonoAVC; IVF-ET; long periodic GnRH antagonist treatment; Oocyte ability; live birth rate

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Introduction

Infertility is one of the most common clinical diseases which would pose serious threat on patients' physical and mental health and family happiness [1]. In terms of medical definition, infertility refers to not being able to become pregnant after a year of having unprotected sex [2].

In vitro fertilization-embryo transfer (IVF-ET), namely test-tube baby, is an artificial method of fertilisation which serves as a major treatment for infertility [3]. In IVF-ET, sperms and unfertilized eggs of the partners are obtained and placed in a controlled environment, where fertilization can take place. The embryo is then placed in the uterus to begin a pregnancy [4-5]. With high pregnancy rate, the effect of IVF-ET technology is remarkable in clinical treatment of infertility. Due to the combined influence of various factors, however, the pregnancy outcome is yet impressionable [6].

Follicle tracking refers to the response to ovarian stimulation assessed by continuous quantification of the number and size of follicle in the IVF process [7], which is a typical two-dimensional (2D) transvaginal ultrasound playing important role in the administration of human chorionic gonadotropin (HCG) before egg retrieval [8]. 2D transvaginal ultrasound assessment, however, becomes more and more difficult and less effective during controlled ovarian hyperstimulation when there are many different sizes of follicle [9]. Three-dimensional sonography-based automated volume calculation (SonoAVC) is a new three-dimensional (3D) ultrasonic diagnostic technology developed in recent years which offers a fast, simple and intuitive new method for accurate and quantitative measurement of low echo objects [10]. This study was conducted in terms of monitoring volume and number of follicles, clinical pregnancy rate and live birth rate compared to 2D ultrasound technology in extensive use currently.

1 Materials and methods

1.1 experimental object

A total of 58 infertile women underwent in vitro fertilization (IVF) in our hospital from January 2019 to January 2020 were selected as subjects, among which 26 cases underwent conventional 2D ultrasonography were assigned to 2D group, aged from 28 to 45 with an average (39.32 ± 2.46) age of years, and 32 cases underwent 3DSonoAVC were assigned to 3D group, aged from 28 to 44 with an average (39.85±2.83) age of years.

Inclusion criteria: 1) The study was approved by the Medical Ethics Committee and patients were well informed of analysis on anonymous clinical data; 2) aged 28 to 45 years; 3) the inability to achieve conception or bring a conception to term after a year or more of regular sexual intercourse without contraceptive protection; 4) patients with presence of both ovaries undergoing long periodic GnRH treatment

Exclusion criteria: Women with 1) polycystic syndrome, hormone-induced infertility; 2) history of oophorectomy and/or partial ovarian resection; 3) severe endometriosis, endometrioma or ovarian masses; 4) history of recurrent (three or more) implantation failures.

1.2 Experimental methods

1.2.1 Ovarian stimulation

Recombinant follicle stimulating hormone (Gonal-f; Merck Serono, Middleton, UK) was used daily to control ovarian hyperstimulation and ovarian response was monitored via transvaginal ultrasound. Pituitary suppression (fixed protocol) were carried out by taking GnRH antagonist (Cetrotides; Merck Serono, Geneva, Switzerland) daily from the fifth day of stimulation, and HCG were recombined (Ovitrelle; Merck Serono, Feltham, England) to triggers final oocyte maturation in the presence of at least 3 primary follicles of 17 mm in diameter (diameter based on average follicle diameter of 2D group and volume reconstruction of 3D group) and oocyte ablation was performed after 36 hours according to local standard operating procedure. Follicle rinsing technology was not considered as an option in our experiment. Embryo transplantation was performed under ultrasound guidance after 48 to 72 h. With luteal support as vaginal progesterone, serum human chorionic gonadotropin (HCG) levels were measured 12 to 13 days after embryo transplantation, patients with positive outcomes underwent ultrasound examination of fetal heartbeat 8 to 12 weeks after surgery, and clinical pregnancy rate and clinical pregnancy frequency were calculated.

1.2.2 Transvaginal ultrasound

All patients were examined by the same investigator and both ovaries were analyzed. Voluson E8 expert ultrasound system were employed in ultrasound technique at the beginning of the menstrual cycle (GE Medical Systems, Chicago, IL, USA) equipped with transvaginal volume transducer to determine the number and volume of antral follicles. Ovarian volume was calculated according to ovarian diameter in the equation of Length × Width × Depth × 0.523. The criteria of ultrasound data acquisition for all subjects were as follows: transducer frequency: 7.5 MHz; gain: −5; enhancement: 2; speckle reduction image: 2; rejection level: 25; harmonic wave: high. Follicle number in the lateral and vertical plane of 2D group were measured with average diameter calculated, the size of antral follicle was determined by two vertical diameters. In the 3D group, the

follicle number was measured and the follicle volume was automatically calculated based on the echo-free follicle region in the scanner. And the device was only employed to analyze interested regions without extraovarian structures, the follicle images were displayed with corresponding observed value and volume, and time of follicular counting in 2D and 3D SonoAVC detection were compared.

1.3 Follicular puncture, observation of oocyte maturity, fertilization, cleavage block method

1) 2~3 follicles of different diameters were taken from the subjects with single follicle puncture, and follicular fluid were collected separately. In this study, 58 follicles with only one oocyte-corona-cumulus complex (OCCC) were selected from the follicular fluid, with 26 follicles in 2D group and 32 in 3D group. 2) The follicle diameter in 3D group were measured before egg retrieval and divided into two groups according to the diameter. Group A was large follicle group with a total of 19 follicles of 16 mm in diameter, group B was small follicle group with a total of 13 follicles of diameter <16 mm.

1.4 Observation indicators

1)Comarison of basic data in 2D group and 3D group; 2) Comarison of left, right and double ovarian volume in 2D group and 3D group; 3) Comarison of follicle number and follicle counting time in 2D group and 3D group; 4) Comarison of group clinical outcomes containing pregnancy rate, live birth rate in 2D group and 3D group; 5) comparison of oocyte maturity, OCCC score, fertilization, cleavage rate in follicles with different diameters; 6) Factors affecting GnRH antagonist IVF cycle treatment were explored using Logistic regressive analysis model.

1.5 Standard of OCCC, fertilization and cleavage score

OCCC scoring standard[11]: Grade I, very thin cumulus and/or radiation crown surrounding the zona pellucida, with first polar body visible; grade II, medium-sized cumulus structure, loose cells, clear boundary of the radioactive crown; Grade III, large and loose cumulus structure , and unclear boundary of the radioactive crown; with Grades I, II, and III rating score of 1, 2 and 3 respectively. Grade II and III were defined as mature oocytes. Single egg fertilization and single embryo culture were performed, and the fertilization and cleavage of each oocyte were recorded. Fertilization scoring standard: Refer to score of pronuclear embryos proposed by Scott [12], Z1, Z2, Z3, Z4 were scored as 4, 3, 2 and 1 point respectively, and 0 point denotes infertilized. Cleavage scoring standard: According to the scoring standard proposed by Bourn Hall Clinic [13] in the UK, the embryos were divided into 1, 2, 3, 4, 5, and 6 grades, corresponding to scores of 6, 5, 4, 3, 2 and 1 respectively, with no cleavage scores 0 point.

1.6 Statistical analysis

Statistical analysis was performed using SPSS version 18.0 software, in which independent-sample T test was applied in group comparison, enumeration data was presented in terms of percentage and examined using chi-square test, and measurement data was presented in terms of (±S). A p value less than 0.05 was considered as statistical significance. Univariate and multivariate analysis of pregnancy outcomes in assisted reproductive technology affecting 3DSonoAVC were performed using Cox regressive model. Statistical significance was considered when p value <0.05.

2 Results

2.1 Comparison of basic data

As shown in Table 1, no differences in such factors as age, body mass index and blood pressure were found between 2D and 3D group (P>0.05).

Table 1 **Comparison of basic data ($\bar{x}$±sd) [n (%)])**

Clinical indicators	Group 2D(n=26)	Group 3D(n=32)	t/X2	P
Age of years	39.32±2.46	39.85±2.83	0.046	0.685
Body index mass(kg/m2)	20.63±2.58	21.22±2.41	1.354	0.178
Systolic pressure (mmHg)	126.47±12.39	128.38±8.25	1.032	0.304
Diastolic pressure(mmHg)	74.53±3.58	73.68±5.39	1.076	0.284
Cause of infertility			0.031	0.861
Salpingemphraxis	12(46.15)	14(43.75)		
Ovulation failure	6(23.08)	8(25.00)		
Oligo-atheno-spermia	4(15.38)	5(15.63)		
Unknown cause	2(7.69)	3(9.38)		
Others	2(7.69)	2(6.25)		
Endometrial thickness **(mm)**	9.27±2.01	9.56±2.11	0.049	0.976

2.2 Comparison of left, right and both ovarian follicle volume

The average volume of left, right and double ovarian follicles in 2D ultrasonic technology were 6.38±1.74ml, 6.08±1.82ml and 6.29±1.81ml, respectively. The average volume of left, right and double ovarian follicles in 3DsonoAVC were 7.28±3.78ml and 7.16 ±3.66ml and 7.22±3.73ml respectively, there existed significant differences between two ultrasound techniques in detecting follicular volume (P<0.05), See Figure 1 for details.

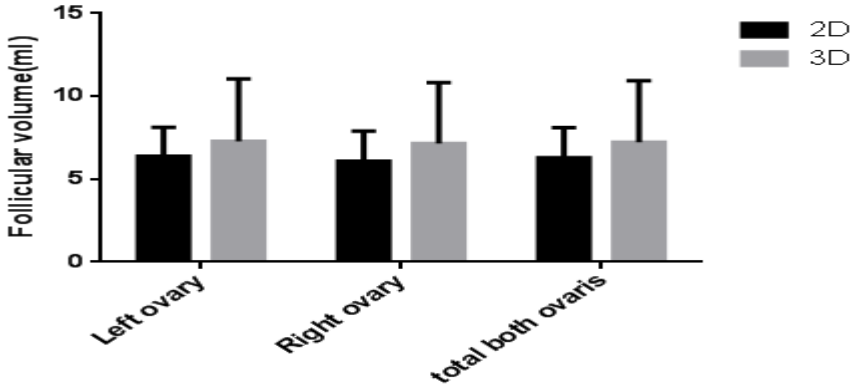

Figure 1. **Volume analysis of left, right and both ovarian follicles.**

Note: T=4.719, P<0.001 in the comparison of left ovarian follicle volume, t=4.046, P=0.015 in the comparison of right ovarian follicle volume, and t=5.126, P<0.001 in the comparison of average volume of both ovarian follicles.

2.3 Comparison of follicle number and follicular count time

A total of 13 follicles (50.00%) were detected using 2D ultrasound technology with consumed time of 283.27±88.13s, and a total of 20 follicles (62.50%) were detected using 3DSonoAVC with consumed time of 104.27±61.23s. Significant difference exhibited in the comparison of follicle number and follicular count time between two different ultrasonic technologies (P<0.05), as shown in Figure 2.

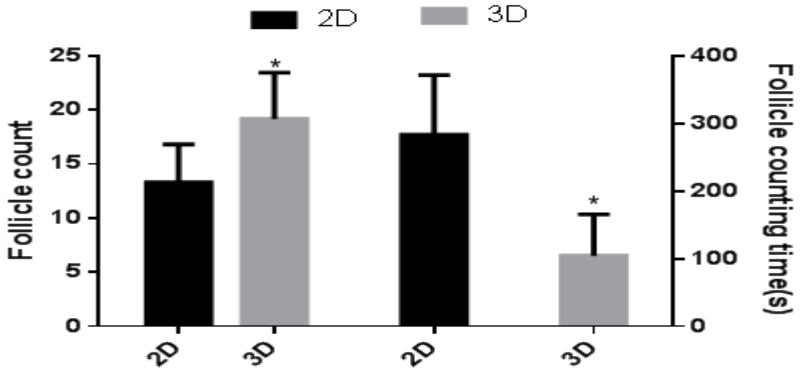

Figure 2. **Analysis of follicle number and follicle count time by two ultrasound techniques.**

Note: T = 16.615, P < 0.001in the comparison of follicle number,t = 9.106, P < 0.001 in the comparison of follicular count time.

2.4 Comparison of pregnancy rate and live birth rate in 2D and 3D groups

As shown in the data of Table 2, there was no difference in pregnancy rate and live birth rate between the 2D and 3D groups (P>0.05), indicating that 3DSonoAVC had no effect on GnRH antagonist IVF periodic treatment.

2.5 Comparison of oocyte maturity and OCCC, fertilization and cleavage score of follicles withdifferent diameters

As shown in the data of Table 3, the proportion of mature oocytes in group A was significantly higher than that in group B (P<0.05). OCCC, fertilization and cleavage score of follicles in group A were significantly higher than those in group B (P<0.05), suggesting that the size of the follicle diameter could exert effect on the clinical pregnancy outcome.

Table 2. **Comparison of pregnancy rate and live birth rate in 2D and 3D groups**

Group	Cases	Pregnancy rate	Live birth rate
Group 2D	26	20(76.92)	19(73.08)
Group 3D	32	25(78.16)	25(78.16)
χ2		1.030	0.916
p		0.308	0.142

Table 3. **Comparison of oocyte maturity and OCCC, fertilization and cleavage score of follicles withdifferent diameters (x±sd) [n(%)]**

Group	Cases	Mature oocyte	Cleavage score	OCCC score	Fertilization score
Group A	19	14(73.68)	4.32±0.61	1.76±0.44	3.04±0.76
Group B	13	5(38.46)	2.71±0.73	1.30±0.43	1.71±0.66
χ^2		28.030	6.346	5.246	7.143
p		<0.001	<0.001	<0.001	<0.001

Table 4. **Univariate analysis on factors affecting pregnancy outcomes of assistedreproductive technology in 3DSonoAVC technology**

Clinical indicators	Pregnant group	Non-pregnant group	P value
Age of years	25	7	<0.001
≤35	5(20.00)	6(85.71)	
＞35	20(80.00)	1(14.29)	
Infertility years			<0.001
≤3	19(76.00)	2(28.57)	
＞3	6(24.00)	5(71.43)	
Cause of infertility			<0.001
Salpingemphraxis	11(44.00)	3(42.86)	
Ovulation failure	6(24.00)	2(28.57)	
Oligo-atheno-spermia	4(16.00)	1(14.29)	
Unknown cause	2(8.00)	1(14.29)	
Others	2(8.00)	0(0.00)	
Endometrial thickness **(mm)**			<0.001
<8	4(16.00)	5(71.43)	
8~16	21(84.00)	2(28.57)	
Transplanted embryo quality			<0.001
excellent	20(80.00)	2(28.57)	
poor	5(20.00)	5(71.43)	

Table 5. **Multivariate analysis on factors affecting pregnancy outcomes of assisted reproductive technology in 3DSonoAVC technology**

Clinical indicators	regression coefficient	standard error	Waldvalue	HR	95%CI	P
Age (≤35 years，＞35 years)	0.064	0.032	6.159	1.067	0.003-1.135	0.041
Infertility duration (≤3 years，＞3 years)	0.217	0.131	1.951	1.342	0.036-1.140	0.066
Endometrial thickness (<8mm，8~16mm)	0.466	0.165	7.926	1.593	1.152-2.204	0.005
Transplanted embryo quality (excellent, poor)	-1.511	0.690	4.789	0.221	0.057-0.854	0.029

2.6 Analysis of factors affecting the pregnancy outcome of assisted reproductive technology with 3DSonoAVC technology

The factors affecting the pregnancy outcomes of 3D group were analyzed by Cox regressive model, in which the results of single factor analysis showed

that age, infertility, endometrial thickness and quality of transplanted embryos were correlated with while the cause of infertility has nothing to do with the pregnancy outcome of assisted reproductive technology in SonoAVC (P<0.05) , and the multi-factor analysis (both P<0.05) showed that infertile patients with transplanted embryo of excellent quality and 8 mm ≤ endometrial thickness ≤ 16 have a good pregnancy outcome in the detection of assisted reproductive technology in SonoAVC. See Table 4-5 for details.

3. Discussion

It is estimated that as many as 186 million people suffer from infertility worldwide [14]. Although male infertility accounts for more than half of childless cases all over the world, infertility remains a social burden for women [15]. Unfortunately, the regions with the highest rate of infertility have found it most difficult accessing assisted reproductive technologies. In this case, women have to live the rest of life without descendants[16].

Since Edward declared the birth of the first test-tube baby in 1978, IVF-ET technology has been widely carried out and greatly developed worldwide with an elevated success rate [17]. China began to conduct research on IVF-ET technology in the 1980s [18]. In 1988, the birth of IVF in the Third Hospital of Beijing Medical University marked a milestone of China's reproductive technology [19]. According to statistics, infertility patients in China currently account for about 13.00% of the childbearing population, of which the rate is still soaring up. With the continuous advancement of IVF-ET technology, infertility problems are readily tackled and solved step by step [20].

Ultrasound examination plays an irreplaceable role in the clinical practice of obstetrics and gynecology. With the rapid development of modern science and technology, constantly innovation was witnessed in ultrasonic diagnostic technology [21]. 3D SonoAVC is a more efficient and accurate method for measuring number and volume of follicle than real-time 2D technology [22]. Our study observed significant differences in terms of average volume of left, right, and double ovarian follicles between 3D (7.28 ± 3.786 ml, 7.16 ± 3.66 ml, 7.22 ± 3.73 ml) and 2D (6.38 ± 1.74 ml, 6.08±1.82 ml, 6.29±1.81 ml) ultrasonic testing(P<0.05). Scholar Fagundes[23] determined that 3D SonoAVC can provide follicular volume measurement with high-accuracy, while the follicular volume calculated according to the equation in 2D ultrasound measurement features poor accuracy. And according to the report of Hernández[24], the accuracy of 2D ultrasound measurement would decrease with elevated follicular volume, while 3DSonoAVC still maintains relatively stable accuracy and is independent from the actual follicular volume, probably given the volume in 3DSonoAVC detection is calculated from all the voxels from reconstructed 3D ultrasound image without relying on simple calculations and inferences of mathematical formula. Meanwhile, scholar Wertheimer [25] documented that 3DSonoAVC can also measure the number of follicles quickly and efficiently for stimulating ovaries with multiple follicular developments, which reduces the time required for ultrasound examination. Our study confirmed that the total number of follicles was significantly less and the time was significantly longer in 2D ultrasonic detection than that of the 3D group (P < 0.05), which was consistent with the above report.

Since the 3D SonoAVC measurement is accurate and effective in stimulating the number of follicle and the follicular diameter in the ovary, we tempted to discuss the clinical changes resulted from 3DSonoAVC guiding the timing of HCG injection in depth. In this study, follicles of different diameters were divided into group A (follicle diameter ≧16mm) and B (follicle diameter <16mm) and the result of our study revealed that the proportion of mature oocytes in group A was significantly higher than that in group B (P<0.05). Moreover, the follicular OCCC score, fertilization and cleavage rate of group A were significantly higher than those of group B (P<0.05). Thus, 3DSonoAVC is competitive to 2D real-time ultrasound currently in extensive use no matter in terms of volume, number or diameter of the follicles in the ovary stimulated by GnRH antagonist. With the increase of follicle number, however, the consistency of 3DSonoAVC and 2D ultrasound measurement would decrease. Certain adjustment for criteria is a necessary in term of the timing of HCG injection by 3DSonoAVC, which does not affect the pregnancy outcome of IVF-ET treatment [26].

The success rate of human reproduction depends largely on the age of women planning for pregnancy, which is soaring up worldwide. And fertility declines as women ages, while the incidence of abortion and important chromosomal abnormalities would increase in contrast. The most important influential factor in IVF on pregnancy rate is yet to be determined, most studies believe that age is the main factor affecting the success rate of assisted pregnancy [27]. In this study, the factors affecting the pregnancy outcome of assisted

reproductive technology in 3DSonoAVC were analyzed by univariate and multivariate analysis, and it was found that the age, infertility, endometrial thickness, and embryo quality were related to the pregnancy outcome of assisted reproductive technology with 3DSonoAVC technique. (P<0.05), and the multi-factor analysis (both P<0.05) showed that infertile patients with transplanted embryo of excellent quality and 8 mm ≤ endometrial thickness ≤ 16 have a good pregnancy outcome in the detection of assisted reproductive technology in SonoAVC. Steiner [28] and other scholars have discovered that women's fertility declines significantly in their 30s, increasing the possibility of infertility. With age >30 years, women who have never been pregnant features a lower probability of becoming pregnant. The main causes of age-related infertility include decreased ovarian reserve and oocyte/embryonic capacity due to aging, especially for that increased incidence of aneuploidy and decreased mitochondrial activity would contribute to the content decline of cytosolic adenosine triphosphate (ATP) and increased apoptosis in the egg, reducing egg number, egg quality and embryo quality, thus leading to the slump of clinical pregnancy rate, and this viewpoint is consistent with the conclusions of our study. A certain endometrial thickness can achieve a higher pregnancy rate, endometrial thickness and volume have a strong negative predictive value for pregnancy outcomes. As a result, correct evaluation of endometrial receptivity, selection of transplantation timing is a key link to improve embryo implantation rate [29]. In the research of scholar Fang [30], 756 cycles were devided into three groups: Group 1 (endometrial thickness <8 mm), Group 2 (endometrial thickness 8-14 mm), and Group 3 (endometrial thickness > 14 mm) . Compared with Group 2 and Group 3, the rate of clinical pregnancy, embryo implantation and live birth of Group 1 were significantly lower (P < 0.001). A higher clinical pregnancy rate was obtained with endometrial thickness ≥ 8 mm, and no adverse pregnancy outcomes would occur with endometrial thickness> 14 mm. Endometrial thickness can be used as an independent predictor of pregnancy outcome. Transvaginal ultrasound monitoring of endometrial thickness before IVF-ET enjoys significant guiding value for choosing best embryo transplantation time.

However, there are some limitations in this study. First of all, the sample size of our experiment is still insufficient. Secondly, blastocyst transplantation was not included in the study, which attenuate the referential value of our study. In order to obtain more fully reliable evidence of evidence- based medicine, larger sample size is a necessity in the following research.

In summary, assisted reproductive technology in SonoAVC showed no better pregnancy outcomes than conventional 2D ultrasonography. Age, embryo quality and endometrial thickness of HCG day were independent factors influencing clinical pregnancy outcome. The clinical pregnancy rate of patients with age ≥35 significantly reduced. Endometrial thickness of HCG day ranged from 8 to 16 mm could improve clinical pregnancy rate.

Conflict of interest

The authors declare that they have no conflict of interest.

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