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Research Article

A Comparison of Fertilization Strategies for Women Undergoing IVF Treatment with Three to Five Oocytes: A Single Centre Analysis of 462 Patients

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Abstract

The study aimed to compare two insemination strategies in women undergoing *in vitro* fertilization (IVF) with three to five retrieved oocytes. A total of 462 patients were recruited in our center and obtained three to five oocytes during their first oocyte retrieval between January 2020 and December 2023. Patients whose oocytes underwent overnight insemination were assigned to group A; while those whose oocytes subjected to short-time insemination (4-6h) were recruited as group B. The clinical pregnancy rate, implantation rate, miscarriage rate and multiple pregnancy rates were similar between the two groups ($P>0.05$). However, group B achieved a higher number of top-quality D3 embryos, a better blastocysts rate, and more frozen embryos compared to group A ($P<0.05$). The early rescue intracytoplasmic sperm injection (ICSI) rate was significantly higher in group B (6.49% Vs 0.00%, P=0.000), resulting in a notably lower total fertilization failure (TFF) rate in group B (2.16% Vs 7.36%, P=0.009) compared to group A. Short-time insemination strategies significantly increase the rate of available embryos rate and decrease the TFF rate, which may be increases the cumulative live birth rate in IVF treatment. This approach may be particularly suitable for patients with three to five oocytes and a favorable clinical prognosis.

INTRODUCTION

With the development of assisted reproductive technology (ART) and the continuous postponement of childbearing age, the incidence of infertility is increasing year by year [1], and patients difficulties are becoming higher and higher, such as Patients' average age becomes older, recurrent implantation failure, premature ovarian insufficiency patients (POI) [2]. In early ART treatment, majority IVF center used over-night fertilization, but more and more studies have found that over-night fertilization leads to potential oxidative stress and damage to oocytes potential due to excessive production of reactive oxygen species by a large number of sperm, resulting in a decrease in the formation rate of high-quality embryos and impaired implantation potential [3]. Moreover, about 1% to 5% patients are suffered total fertilization failure (TFF) after overnight fertilization [4]. Therefore, some scientists have proposed removing granulosa cells 1-6 hours after sperm-oocyte fusion in order to reduce oxidative stress on oocytes, and further it can timely perform rescue intracytoplasmic sperm injection (ICSI) if occurred TFF, which may be achieved much better clinical outcomes for patients than over-night rescue ICSI [5]. Recent years, more and more reproductive centers have adopted short-time insemination [6]. In clinical practice, there is some confusion about whether short-time insemination is necessary for oocyte numbers ≤5, especially when judging in cases two polar bodies are not obvious, fragmented, or even at the borderline ratio, which brings some confusion to clinical work [7]. Therefore, there are few reports on whether short-time insemination is necessary for ≤5 oocytes [8].

This study aim to compare and analysis the embryo and clinical pregnancy outcomes between the over-night insemination group (Group A) and the short-time insemination group (Group B) for the number of oocytes between 3 to 5.

MATERIALS AND METHODS

Patients

Patient who visited the Reproductive Medicine Center

of Shenzhen Hengsheng Hospital from January 2020 to December 2023, which were divided into Group A and Group B based on the sperm-oocyte co-incubation time. The recruited patients met the following inclusion and exclusion criteria: the first oocyte retrieval in our center, the number of retrieved oocytes ranging from 3 to 5; semen from the male partner meets the IVF fertilization criteria. The study was approved by the Institutional Reviewer Board of the hospital (20240227), and informed consent was obtained from all participants.

Controlled Ovarian Hyper-Stimulation (COH) Protocol

Some protocols were used, including mini cycle, luteal phase stimulation; progestin primed ovarian stimulation (PPOS), modified natural cycle. Oocyte retrieval was performed 36h after human chorionic gonadotropin (hCG) administration, and insemination were carried out 39h after hCG administration.

Embryology Laboratory Procedures and Morphology Assessment

All culture media and oil products were purchased from Vitrolife, Sweden. Embryo culture was performed at 37°C, 6% CO_2, 5% O_2, in a humidified atmosphere. *In vitro* fertilization was performed using the overnight insemination group (Group A) or the short-time insemination group (Group B). Within 16 to 18 hours after insemination, the zygote with two pronuclei was regarded as the normally fertilized. Morphologic parameters for cleavage embryos mainly included the number and symmetry of blastomeres (cell-stage specific), and the percentage of fragmentation, multi-nucleation, vacuoles, endoplasmic reticulum, and morphologic of zona pellucida [9]. Blastocysts showing an expansion from 3 to 6 with quality inner cell mass and TE grade A, B, or C were eligible for scoring on day 5 or 6 according to the method of Gardner scoring [10].

Primary and Secondary Outcomes

The primary outcomes were the early rescute ICSI rate and TFF rate in two groups. The secondly outcomes included the normal fertilized rates, number of available embryos and clinical pregnancy rates.

Statistical Analysis

Variables were expressed as means and standard deviations. Categorical variables were expressed as absolute values and percentages. Differences were considered statistically significant if $P<0.05$. Quantitative variables were compared by Student's *t*-test or the Mann-Whitney U test, as appropriate. Paired statistics were compared by the Wilcoxon rank test. Categorical variables were compared by the chi-squared or Fisher's exact test. Statistical analysis was performed with the statistical package Statistical Package for the Social Sciences (SPSS)16.0.

RESULTS

The study comprised 462 eligible patients, and patients were randomly divided into two groups. Table 1 displays patients' basic clinical data, which were similar between two groups regarding the female age, male age, duration of female infertility, etiology, BMI, basal FSH, AMH, and the number of antral follicle count (AFC) (P>0.05).

Table 2 presents comparisons of embryo parameters in two groups. The number of oocytes retrieved, normal fertilization rate, multiple pronucleus fertilization rate, and normal cleavage rate were similar between groups(P>0.05). However, group B exhibited significantly higher numbers of day 3 top embryos, blastocyst rate, available embryos, and frozen embryos(P<0.05). Conversely, the early rescue ICSI rate was dramatically higher in group B, leading to a significantly lower TFF rate compared to group A (P<0.05).

Table 1: Comparison of patients' basic parametres

	Group A	Group B	*P* value
n	231	231	
female age (years)	38.00±5.01	38.40±4.44	0.366
male age (years)	39.00±6.02	39.36±6.16	0.522
female infertility (years)	3.93±3.18	4.30±3.96	0.269
aetiology			
low ovarian reserve	68(29.44)	84(36.36)	0.437
ovulatory dysfunction	9(3.89)	9(3.89)	0.968
Tubal factor	108(46.75)	110(47.62)	0.573
endometriosis	29(12.55)	19(8.23)	0.384
male factor	6(2.59)	1(1.73)	0.876
unexplained infertility	4(1.73)	1(1.73)	0.985
mutliple factor	8(3.46)	8(3.46)	0.978
BMI (kg/m2)	21.96±2.82	22.30±2.82	0.207
AMH (ng/ml)	1.51±1.06	1.52±1.04	0.92
Basal FSH(IU/L)	8.10±4.10	7.41±3.69	0.602
AFC	7.33±4.24	7.35±3.98	0.976

Table 2: Comparison of Embryo Data

	Group A	Group B	P value
n	231	231	
Oocyte numbers	3.97±0.82	3.94±0.80	0.648
Early rescute ICSI rate	0.00(0/231)	6.49(15/231)	0.000
Total fertilization failure	7.36(17/231)	2.16(5/231)	0.009
Normal fertilized rate	63.40(582/918)	64.25(584/909)	0.706
Multiple pronuclei rate	7.41(68/918)	7.92(72/909)	0.681
Cleavaged embryos rate	99.14(577/582)	100.00(584/584)	0.052
Day 3 top embryos rate	42.46(245/577)	52.74(308/584)	0.013
Blastocysts formation rate	41.01(89/217)	51.99(144/277)	0.009
Available blastocysts rate	66.29(59/89)	72.92(105/144)	0.282
Number of frozen embryos	1.51±1.16	1.78±1.14	0.027

Notably, patients in group B had more embryos available for transfer(P<0.05), increasing their chances of embryo transfer.

Clinical analysis revealed that patients in group B achieved similar pregnancy rates compared to group A for each transfer cycle, with no statistically significant differences between the two groups(P>0.05). Additionally, there were no significant differences in other clinical outcomes, including implantation rate, live birth rate, early miscarriage rate between two groups, as shown in Table 3 (P>0.05).

DISCUSSION

Short-time insemination is increasingly favored by more and more reproductive centers [11]. The mainly advantage of short-time insemination is to avoid complete fertilization failure [12]. This study analyzed and studied the patient with the number of 3 to 5 oocytes. The experimental results showed that there was no statistically significant difference in clinical outcomes between the Short-time insemination group and over-night insemination group, but the TFF rate were significantly lower and the embryo utilization rate were obviously higher in the short-time insemination group, providing patients with more clinical transfer opportunities. Furthermore, with an increased sample size, the cumulative clinical pregnancy rate of the short-time insemination group may be significantly higher.

Observation of the second polar body usually occurs 2-6 h and observation of normal fertilization often 17±1 h after sperm-egg fusion. In early development of ART, embryologist often performed conventional over-night insemination. The biggest flaw for overnight fertilization is suffered TFF, which might be affected about 1%-5% patients, and the clinical outcomes were significantly decreased even though embryologist performed late rescue ICSI [13]. Therefore, some scientists explored short-time insemination protocols, which remove granulosa cells after sperm-oocyte fusion 2-6 h, fertilization can be judged by the proportion of second polar bodies in mature oocytes [12]. If the patient encounter completely or partially fertilization failure, we are promptly performed early ICSI for rescue, and numerous data have confirmed that the clinical pregnancy rates are greatly improved compared to late rescue ICSI [14], and the clinical outcomes after early ICSI rescue are similar to those of conventional IVF/ICSI clinical outcomes [15]. For this reason, more and more fertility centers are adopting short-time insemination protocols for IVF treatment. In our study, the results indicated that the clinical pregnancy rates, implantation rates, early miscarriage rates, and multiple pregnancy rates are similar to those in the overnight fertilization group, with no significant differences between the two groups. This suggests that the clinical pregnancy outcomes are not correlated with either short-time or overnight fertilization. Furthermore, we found no significant difference in polyspermy rates between the short-time and overnight fertilization groups, indicating that short-time insemination does not significantly increase the incidence of polyspermy. Even though some studies have found that the short-time insemination increases the proportion of polyspermy in the short-time insemination group [16].

Table 3: Comparison of Clinical Outcome

	Group A	Group B	P value
n	231	231	
female age (years)	36.54±5.01	37.72±4.44	0.366
Number of transferred cycles	72	56	/
Number of transfer embryos	1.90±0.35	1.70±0.46	0
HCG positive rate	58.33(42/72)	62.50(35/56)	0.633
Clinical pregnancy rate	54.17(39/72)	51.79(29/56)	0.724
Implantation rate	35.04(48/137)	33.68(32/95)	0.831
Multiple rates	23.08(9/39)	10.34(3/29)	0.173
Early miscarriage rate	15.38(6/39)	17.24(5/29)	0.837
female age (years)	36.54±5.01	37.72±4.44	0.366

Furthermore, our study revealed that the total embryo quality and the blastocyst formation rates were higher in short-time insemination group, demonstrating that the short-time insemination might enhances both the developmental potential and utilization rate of embryos. Secondly, the number of available embryos for cryopreservation were significantly higher in the short-time insemination group, which might be increasing the times of embryo ET and increasing the cumulative clinical pregnancy rate after early rescue ICSI [17]. Additionally, another advantage of short-time insemination is the ability to promptly perform early rescue ICSI in cases of majority or totally failed fertilization, reducing the likelihood of complete fertilization failure cycles. furthermore, our study showing that early ICSI rescue can increase the efficiency of oocyte utilization, providing patients with more opportunities for conception.

In summary, for patients with 3-5 oocytes, the short-time insemination approach ought to be considered. It does not affect the clinical pregnancy outcome of each transfer cycle but can offer two advantages: firstly, it significantly improves the number and overall quality of embryos after short-time insemination, secondly, it performs early ICSI rescue for patients with fertilization failure, thereby enhancing the patients' oocyte utilization rate and improving their cumulative clinical pregnancy outcomes.

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