Day 3 embryo transfer may have better pregnancy outcomes in younger than 35-year-old patients with poor ovarian response

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Abstract

Objective To explore the pregnancy outcomes of embryo transfer with D2 or D3 embryos in patients with poor ovarian response.

Methods The pregnancy outcomes of 620 patients who had poor ovarian response and underwent the first in vitro fertilization-embryo transfer (IVF-ET) were retrospectively analyzed. Of the 620 cycles, all available fresh D2 embryos were used in 365 cycles (day 2 embryo transfer) and all available fresh D3 embryos were used in 255 cycles (day 3 embryo transfer) without superfluous embryos for freezing.

Results There was a significant difference in clinical pregnancy rate between day 2 (32.73 %) and day 3 (50.83 %) embryo transfer in younger than 35-year-old patients, but no significant differences in implantation rate, live birth rate and spontaneous abortion rate ($P>0.05$). There were similar pregnancy outcomes between day 2 and 3 embryo transfer in 35-year and older patients.

Conclusion D3 embryo transfer may have better pregnancy outcomes in younger than 35-year-old patients with poor ovarian response.

Keywords Poor ovarian response · In vitro fertilization · Pregnancy outcomes · All available embryos

Capsule This study suggests that day 3 embryo transfer may have better pregnancy outcomes in younger than 35-year-old patients with poor ovarian response.

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Introduction

With the development of stimulation protocol, cell culture and embryonic selection criteria, the success rate of in vitro fertilization (IVF) is increasing from 20–30 % to 40–50 %. The key to improving the success rate for in vitro fertilization-embryo transfer (IVF-ET) is to obtain adequate numbers of high-quality oocytes and embryos which depend on ovarian reactivity. However, poor ovarian response is still a problem for the present reproductive technology. The treatment cycle rate and cumulative successful rate are decreased due to less or no available oocytes or embryos in the patients with poor ovarian response. In patients with good ovarian response, in vitro embryo culture duration is extended in order to obtain more high-quality embryos. However, in patients with poor ovarian response, there have been conflicting reports regarding the best timing of embryo transfer. It has been reported that the pregnancy outcomes are better in day 2 embryo transfer than in day 3 embryo transfer [1, 2], or similar in day 2 and day 3 embryo transfer [3, 4]. However, in these studies, the definition for poor ovarian response is different, non-initial IVF-ET is also included and live birth rate fails to be reported.

In this study, the pregnancy outcomes of the patients with poor ovarian response who underwent IVF-ET in our Reproductive Center for the first time were retrospectively analyzed to find the best timing of embryo transfer.

Subjects and methods

All study methods were approved by Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects enrolled into the study gave written formal consent to participate.

Subjects

The data of 620 initial IVF-ET cycles preformed in our Reproductive Center between July 2009 and June 2011 were collected. The inclusion criteria included that (1) the patients had poor ovarian response; (2) the patients underwent IVF-ET for the first time; (3) the patients had no smoking history; (4) the patients had normal ovarian reserve (FSH < 15 IU/L on the day 3 menstruation); (5) the patients had normal uterine cavity identified with hysteroscopy and (6) all available fresh embryos were used in embryo transfer and no superfluous embryos were used for freezing. The exclusion criteria were (1) IVF-ET due to male infertility; (2) the use of donor oocytes in IVF-ET and (3) the patients without available embryos for transfer. Of the 620 cycles, D2 embryos were used in 365 cycles (day 2 embryo transfer) and D3 embryos were used in 255 cycles (day 3 embryo transfer).

The assisted reproductive technology specification made by Ministry of Health of China describes that for the patients undergoing IVF for the first time, younger than 35-year-old patients should receive two transferred embryos or less, and 35-year and older patients should receive three transferred embryos or less. In this study, all available fresh embryos were use in each patient without superfluous embryos for freezing, and the number of transferred embryos was in line with the technological specification. The patients with more available fresh embryos were excluded from this study.

Day 2 or day 3 embryo transfer was performed according to the convenience of patients and medical team. Day 2 and day 3 embryo transfer was performed in similar proportions continually over the study period.

Diagnostic criteria for poor ovarian response

There are not unified criteria for poor ovarian response. Different authors have adopted different criteria. For example, the dominant follicles are less than 3 or 5 on the day of HCG injection [5, 6], the number of retrieved oocytes are less than 4 or 6 [7, 8], or the peak value of E_2 is less than 300 pg/ml or 500 pg/ml [9, 10]. In this study, less than 6 retrieved oocytes after routine superovulation protocol was served as the diagnostic criteria for poor ovarian response [8].

Controlled ovarian hyperstimulation (COH)

Patients were stimulated with standard GnRH long protocol. This protocol began with pituitary desensitization using a GnRH agonist (Decapeptyl; Ferring Pharmaceuticals, Kiel, Germany) in the mid luteal phase of preceding menstrual cycle. Administration of gonadotropins (gonal F; Serone, Aubonne, Switzerland) was initiated on day 3 of the

commencing cycle. When the leading follicle reached a diameter of 18 mm or two oocytes a diameter of 17 mm, 10000 IU HCG (HCG, Lizhu, Zhuhai, China) was administered to trigger ovulation. Oocytes were retrieved 34–36 h after HCG injection, and then were subjected to IVF.

Embryo culture and transfer

Oocytes and embryos were incubated with the medium provided by Vitrolife Company (Switzerland) and the semen was treated by density gradient centrifugation.

Oocytes were washed, then incubated in a mixed atmosphere of 5 % CO_2 , 5 % O_2 and 90 % N_2 . Short-term insemination was performed 39–40 h after HCG injection; 4 h later, the second polar body was observed.

Observation on embryos and embryo-grading criteria

The pronucleus status was observed 16–20 h after insemination, and two pronuclei (2PN) were regarded as normal fertilization. Embryo transfer was performed 48 h or 72 h after ovum pick-up. All obtained embryos were evaluated according to the embryo-grading criteria [11], and grade I and II are regarded as high-quality embryos. Grade I–III embryos were used in embryo transfer.

Embryo-grading criteria were (1) grade I: uniform blastomere with intact zona pellucida and less than 10 % fragment; (2) grade II: slightly nonuniform blastomere and less than 10 % fragment; (3) grade III: blastomere as that in grade II with intact zona pellucida and less than 50 % fragment; (4) grade IV: blastomere being viable and more than 50 % fragment; (5) grade V: 2PN in Day 2, or delayed fertilization; (6) grade VI: inviable embryo, blastomere lysis.

Conditions in clinic and embryo laboratory

There were no marked changed in laboratory or clinical protocols, medical team, and the number and distribution of patients between July 2009 and June 2011.

Diagnosis of clinical pregnancy

It was diagnosed as clinical pregnancy that B-mode ultrasound showed embryo sac 35 days after embryo transfer.

Statistical analysis

Statistical analysis was performed with SPSS17.0 software. t test was used for the comparison between groups. χ^2 test was used in qualitative data analysis. Relative risk (RR) and 95 % confidence interval were used to evaluate the influence of embryo transfer timing on pregnancy outcomes. In order

to identify the effects of embryo transfer timing on pregnancy outcomes, the relative risk (RR) was calculated according to exposure group (day 3 embryo transfer was served as control group). RR was used for the comparisons of implantation rate, clinical pregnancy rate, live birth rate and spontaneous abortion rate between groups, and the 95 % confidence intervals (CI) were calculated. Statistical significance was established at $P < 0.05$.

Results

Comparison of pregnancy outcomes between day 2 and day 3 embryo transfer in younger than 35-year-old patients

There was a significant difference in clinical pregnancy rate ($P < 0.05$) between day 2 and day 3 embryo transfer in younger than 35-year-old patients, but no significant differences in implantation rate, live birth rate and spontaneous abortion rate ($P > 0.05$) (Table 1).

Comparison of pregnancy outcomes between day 2 and day 3 embryo transfer in 35-year and older patients

There were no significant differences in pregnancy rates, implantation rate, live birth rate and spontaneous abortion rate in the 35-year and older patients ($P > 0.05$) (Table 2).

Discussion

Poor ovarian response is mainly due to decreased ovarian reserve, so the assessment of ovarian reserve is often used to predict ovarian reactivity. Poor ovarian response can be diagnosed according to several indexes, such as serum E_2 , doses of ovulation stimulants, the number of development-

effective ovarian follicles or the number of retrieved oocytes. However, the assessment for ovarian reserve is all indirect, so unexpected poor ovarian response has considerable influence on IVF. Bronco et al. [12] have believed that it is the most direct strategy to use the number of retrieved oocytes to evaluate poor ovarian response. It is reported that in different assisted reproductive methods, the number of retrieved oocytes which is used to diagnose poor ovarian response should be different, and they are 5 in intracytoplasmic sperm injection (ICSI), 6 in IVF and 8 in testicular sperm extraction (TESE) combined with ICSI, respectively [12]. In this study, less than 6 retrieved oocytes was served as the diagnostic criteria for poor ovarian response.

With the increase in age, the number and quality of oocytes and reproductive function gradually decrease, especially in 35-year-old women. In this study, 35-year and older women accounted for 53.6 % of all patients with poor ovarian response. At the same time, we also noticed that younger than 35-year-old women accounted for 46.4 %, so a great deal of attention also should be paid to younger patients with poor ovarian response in clinical practice.

It has been indicated that in vitro developmental arrest of human embryos mainly occurs in the stage of 4 to 8 cells [13]. Therefore, the developmental arrest can be observed in D3 embryos, while it is difficult to evaluate developmental arrest in D2 embryos. In this study, the difference in high-quality embryos between day 2 and day 3 embryo transfer suggests that developmental arrest may occur in partial D3 embryos, which leads to lower embryo scoring; but their similar pregnancy outcomes suggest that the developmental arrest in D3 embryos fails to affect pregnancy rates. Laverge et al. [14] revealed that although the high-quality embryo rate was higher in day 2 embryo transfer than in day 3 embryo transfer, there were no significant differences in pregnancy rate and implantation rate between day 2 embryo transfer and day 3 embryo transfer, which is similar to our results.

Table 1 Comparison of pregnancy outcomes between day 2 and day 3 embryo transfer in less than 35-year-old patients

	Day 2 embryo transfer	Day 3 embryo transfer	P values
Cycles (n)	168	120	
Age (year)	30.8±2.41	30.1±2.28	0.823
High-quality embryos (%)	82.05(320/390)	60.92(290/476)	0.000
FSH(IU)	9.26±2.67	8.58±2.78	0.265
E_2 Peak value (pg/ml)	2210.38±1169.56	2590.29±1168.70	0.856
No. of retrieved oocytes(n)	2.98±1.59	4.02±0.76	0.342
No. of transferred embryos(n)	1.89±0.50	1.96±0.19	0.672
Clinical pregnancy rate (%)	32.73(55/168)	50.83(61/120)	0.046 RR0.65(0.43–0.99)
Implantation rate (%)	24.21(62/256)	33.33(77/231)	0.086 RR0.70(0.49–1.06)
Ongoing pregnancy rate (%)	10.71(18/168)	31.67(38/120)	0.039 RR0.34(0.25–0.96)
Live birth rate (%)	17.85(30/168)	15.0(18/120)	0.716 RR1.23(0.55–2.76)
Spontaneous abortion rate (%)	12.7(7/55)	8.19(5/61)	0.742 RR1.75(0.32–9.62)

FSH follicle-stimulating hormone; E_2 : estradiol

Table 2 Comparison of pregnancy outcomes between day 2 and day 3 embryo transfer in 35-year-old patients or above

	Day 2 embryo transfer	Day 3 embryo transfer	P Values
Cycles (n)	197	135	
Age (year)	37.2±1.58	36.3±1.69	0.791
High-quality embryos (%)	78.18(344/440)	44.28(190/429)	0.000
FSH(IU)	9.28±2.56	9.14±2.31	0.323
E ₂ Peak value (pg/ml)	2391.76±1317.84	2462.89±1204.08	0.404
No. of retrieved oocytes (n)	3.26±1.30	3.98±1.20	0.441
No. of transferred embryos (n)	2.14±0.78	2.28±0.55	0.302
Clinical pregnancy rate (%)	24.87(49/197)	23.7(32/135)	0.610 RR1.21(0.59–2.47)
Implantation rate (%)	15.57(57/366)	11.83(33/279)	0.160 RR1.69(0.80–3.58)
Ongoing pregnancy rate (%)	3.56(7/197)	7.40(10/135)	0.076 RR0.48(0.32–1.10)
Live birth rate (%)	12.18(24/197)	11.11(15/135)	0.809 RR1.10(0.48–9.18)
Spontaneous abortion rate (%)	36.73(18/49)	21.87(7/32)	0.692 RR1.57(0.43–5.77)

FSH follicle-stimulating hormone; E₂: estradiol

In our study, clinical pregnancy rate was higher in day 3 embryo transfer than in day 2 embryo transfer, but there were no significant differences in live birth rate and spontaneous abortion rate between day 3 embryo transfer and day 2 embryo transfer in younger than 35-year-old patients. In our study, some patients have not given birth and are still in gestation now. In younger than 35-year-old patients, 18 patients who underwent day 2 embryo transfer and 38 patients who underwent day 3 embryo transfer are still in gestation now. The live birth rates in Table 1 were from the patients who have given birth, so further follow-up may obtain significant live birth outcome. This study indicated no significant differences in clinical pregnancy rate, live birth rate and spontaneous abortion rate between day 2 and day 3 embryo transfer in 35-year and older patients. Under physiological conditions, the embryos with 4 to 8 cells are located in the oviducts, and then do not enter the uterus until they develop into blastula stage; so compared with D2 embryos, D3 embryos may be closer to the physiological time that embryos enter the uterus [15]. Therefore, we speculate that D3 embryos may be more suitable for younger than 35-year-old patients due to their better oocyte quality and embryonic development potential; but extending in vitro embryo culture duration may have no benefit for more than 35-year-old patients due to decreased quality of oocytes and embryos.

Shen et al. [1] reported that for less than 40-year-old women who had no selectable embryos, D2 embryos could obtain better pregnancy outcomes; which is different from our result that day 2 embryo transfer failed to obtain better pregnancy outcomes in the patients with poor ovarian response. In our study, the subjects underwent IVF for the first time and received 2 or 3 embryos (all available embryos) for embryo transfer; while in Shen et al. [1] study, some subjects underwent several IVF. Different research designs may lead to different results. In Bahecci et al. [2] study, the

number of transferred D2 embryos was higher than that of D3 embryos, which led to higher pregnant rate in day 2 embryo transfer. The differences between our study and Bahecci et al. [2] study are that in our study, research design is retrospective analysis, less than 6 retrieved oocytes is served as the diagnostic criteria for poor ovarian response and all available embryos are transferred; in Bahecci et al. study, poor ovarian response is diagnosed according to the number of dominant follicles. In our study, the patients with poor ovarian response were determined according to the number of retrieved oocytes, which avoids selection bias. We believe that compared with the number of ovarian follicles, the number of retrieved oocytes as diagnostic criteria for poor ovarian response is closer to clinical practice, which is consistent with the viewpoint of Shahine et al. [16].

In summary, there are similar pregnancy outcomes between day 2 and 3 embryo transfer in 35-year and older patients with poor ovarian response. Day 3 embryo transfer may have better pregnancy outcomes in younger than 35-year-old patients with poor ovarian response. This remains to be further confirmed.

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