

Original Article

Prognostic value of three pro-nuclei (3PN) incidence in elective single blastocyst-stage embryo transfer

Mingzhao Li, Wanqiu Zhao, Wei Li, Xiaoli Zhao, Juanzi Shi

The ART Center, Northwest Women's and Children's Hospital, Xi'an 710003, China

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Abstract: Objectives: To investigate whether 3PN incidence could lead to a worse pregnancy outcome of elective single blastocyst stage embryo transfer (eSBET). Methods: This study included 706 eSBET cycles from January 2013 to March 2015. The patients were divided into two groups as follows: group 1 included 286 patients with no 3PN zygotes and group 2 included 420 patients with 3PN zygotes. Main outcome measure: female age, endometrial thickness, infertility duration, type of infertility, main etiology of infertility, clinical pregnancy and abortion rate. Results: We observed that there was no significant difference in female age, endometrial thickness, infertility duration and abortion rate between two groups. It's worth noting that the clinical pregnancy rate in 3PN > 0% group was significantly lower than 3PN = 0 group ($P = 0.021$). Conclusions: 3PN incidence might make a negative effect on pregnancy outcomes in elective single blastocyst stage embryo transfer.

Keywords: 3PN, single blastocyst, pregnancy

Introduction

The delivery of a single healthy child is increasingly perceived as the better outcome for assisted reproductive technology (ART). However, in the past three decades, the twin pregnancy rate of overall population increased almost 50%-70% which might be caused that more IVF centers switched from day-3 cleavage stage to day-5/6 transfers of blastocyst-stage embryos [1]. Some investigators reported that implantation and clinical pregnancy rate after blastocyst stage embryo transfer (BSET) appeared superior to cleavage-stage embryo transfer (CSET) [2]. So the proportion of elective single blastocyst stage embryo transfers (eSBET) in all the BSET cycles was increased to reduce the twin pregnancy rate with years. There is no doubt that if single embryo transferred the clinical pregnancy rate might be lower than transfer with two embryos.

So in eSBET cycles, we need to pay more attention to some factors which may influence the implantation rate. Some studies have suggested that 3PN incidence may serve as a prognostic indicator for IVF cycle outcome using embryos derived from normally fertilized oocytes.

Rosen et al. [3] showed that 3PN formation in this subset of patients is a significant negative predictor of implantation rate. Figueira et al. [4] observed an approximately 50% lower risk of pregnancy and a 3.5-fold higher risk of miscarriage in cycles with a 3PN incidence of > 25%. It suggested that the implantation rate might be affected by 3PN incidence. With this in mind, the aim of this study is to investigate whether 3PN incidence could lead to a worse pregnancy outcome in the eSBET cycles.

Materials and methods

This study was a retrospective analysis of the data from our center and was approved by the Ethics Review Board of Northwest Women's and Children's Hospital. This study included 706 eSBET cycles from January 2013 to March 2015. The patients were divided into two groups as follows: group 1 included 286 patients with no 3PN zygotes and group 2 included 420 patients with 3PN zygotes.

Ovarian stimulation

All patients used the standard long protocols with GnRH agonist (GnRH-a, Decapeptyl Ger-

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Table 1. Comparison of basic characters and clinical outcomes in groups with 3PN = 0 and 3PN > 0%

Parameter	3PN = 0	3PN > 0%	P value
Female age (yr)	28.85±3.70	28.81±3.62	P = 0.747
Endometrial thickness (mm)	11.22±2.43	11.42±2.49	P = 0.559
Infertility duration (yr)	3.23±2.38	3.09±2.31	P = 0.613
Type of infertility (cycles, %)			
Primary infertility	50.70 (145/286)	49.29 (207/420)	P = 0.712
Secondary infertility	49.30 (141/286)	50.71 (213/420)	P = 0.712
Main etiology of infertility (cycles, %)			
Tubal factor	61.19 (175/286)	61.20 (257/420)	P = 0.999
Male factor	11.54 (33/286)	8.81 (37/420)	P = 0.163
Male and female factor	12.94 (37/286)	12.14 (51/420)	P = 0.754
Other reasons	14.33 (41/286)	17.86 (75/420)	P = 0.215
Single blastocyst (total, n)	286	420	/
Clinical pregnancy (n, %)	63.64 (182/286)*	57.62 (242/420)*	P = 0.021
Abortion (n, %)	6.59 (12/182)	7.85 (19/242)	P = 0.622

*P<0.05.

many) and recombinant FSH (GONAL-f, Merck Serono Italy; Puregon, Organon Netherlands) for controlled ovarian hyper-stimulation (COH). 10,000 units of human chorionic gonadotrophin (hCG) were administered when > 3 follicles were > 18 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided aspiration.

Blastocyst evaluation and selection for transfer

Blastocyst evaluation was performed according to Gardner Grade Standard. In this study, eSBET was performed on day 5 and was the first transfer for the conventional IVF patients. The ET catheter (COOK IRELAND LTD, Ireland) was used for transfers. Before transfer, any vaginal and cervical secretions were gently removed from the vagina/cervix with small pledgets of cotton wool, moistened with warm normal saline. The mucus in the cervical canal was wiped away. After transfer, the catheter was checked for retained embryo sand the presence of blood. After ET, all patients were given luteal support (Duphaston; progesterone injection). Clinical pregnancy was confirmed by the presence of a gestational sac [5].

Statistical analysis

Data were analyzed using the SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). When comparing means between different groups, the paired Student's t-test was applied. The χ^2 test was used for group comparison of rate.

Differences were considered statistically significant at P<0.05.

Results

For the entire study, 420 cycles were characterized by the presence of zygotes with 3PN, whereas no incidence of 3PN zygotes was observed in 286 cycles. The distribution of cycle-specific parameters between groups is described in **Table 1** and no significant difference were observed in female's age, endometrial thickness and infertility duration (P > 0.05). The rate of infertility type and main infertility etiology were also no significant difference between two groups (P > 0.05). However, the clinical pregnancy rate in 3PN = 0 group was significantly higher compared with 3PN > 0% group (63.64% vs 57.62%, P = 0.021). The abortion rate was no significant difference between 3PN = 0 group and 3PN > 0% group (6.59% vs 7.85%, P = 0.622).

Discussion

At present, there has been an agreement that blastocysts have higher implantation rates than cleavage stage embryos, however, it also increases the risk of the twin pregnancy rate [6]. As is well known, safety is the common concern of ART technology and it has been a research hotspot in how to reduce the incidence of multiple pregnancy [7]. When selecting embryos by the same criteria, it is no doubt that transferring 1 embryo could not result in an

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equal chance of delivery as transferring 2 equally embryos. So if eSBET performed, it is significant to pay more attention to select the transferrable embryo.

Some studies suggested that if the quality of blastocyst was good enough, the clinical pregnancy rate would not be affected and the multiple pregnancy rate would be reduced compared with transferring 2 blastocysts [8, 9]. In addition to female basal characteristics such as endometrial receptivity and synchronicity, it might also exist other factors affecting the successful pregnancy probability in eSBET cycles. Rosen et al. [3] and Figueira et al. [4] believed that 3PN formation in this subset of patients is a significant negative predictor of implantation. In conventional IVF cycles, the mechanism of polyspermy block was complicated and was effective to ensure normal fertilization occurrence, so 3PN incidence might be caused by oocyte quality or sperm abnormality [10]. Although the blastocyst morphology was better, it might not represent a good ability of implantation.

In this study, the patients were divided into two groups as follows: group 1 included patients with no 3PN zygotes and group 2 included patients with 3PN zygotes. Firstly, we compared some basal parameters of the couples and found no significant difference. Secondly, we observed that the rate of infertility type and main infertility etiology were also no significant difference between two groups. Interestingly, our result showed that the clinical pregnancy rate in 3PN > 0% group was significantly lower compared with 3PN = 0 group (57.62% vs 63.64%, $P = 0.021$) which might suggest that 3PN incidence make a negative effect on implantation. We further compared the proportion of blastocysts with different grade between two groups. In our lab, each blastocyst was evaluated based on the Gardner grading system. However, we observed no significant difference in the proportion of transferrable blastocysts with different grade between two groups ($P > 0.05$). We also obtained the similar results in double blastocyst-stage embryo transfers which showed the implantation and clinical pregnancy rate were significantly higher in 3PN = 0% than 3PN > 20% group ($P < 0.05$). These findings might remind us the existence of 3PN zygotes should be considered if eSBET performed.

It goes without saying that the reasons for 3PN incidence are complicated. Some investigators have suggested that the incidence of 3PN fertilization is a result of advanced maternal age or severe sperm abnormalities [11]. Others have suggested that the propensity toward 3PN is a function of ovarian stimulation which is indicated by high peak E2 levels, large oocyte yields, high gonadotropin doses and lengthy stimulations [12]. Plachot et al. [13] showed a lower incidence of polyspermy when sperm quality was reduced. Levitan et al. [14] showed that egg size has influence on the risk of polyspermy. It is more likely that lower implantation rate in 3PN > 0% group was caused by certain factor. However, we could not divide the group according to the different factors which resulted in 3PN incidence because of the limited cases. So in the future work, it is more important to accumulate clinic data for a more persuasive conclusion.

Disclosure of conflict of interest

None.

Address correspondence to: Juanzi Shi, The ART Center, Northwest Women's and Children's Hospital, Xi'an 710003, China. E-mail: szzxsjz@163.com

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