Effect of Blastocyst Quality on Human Sex Ratio at Birth in Single Blastocyst Frozen Thawed Embryo Transfer Cycle

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Research

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Abstract

Objective

This study aimed to determine whether blastocyst quality affect on sex ratio at birth through in single blastocyst frozen thawed embryo transfer cycle.

Materials and Methods

We ran this retrospective study on 4205 singleton infants born following single blastocyst frozen thawed embryo transfer from January 2016 to October 2020 at a single institution. We compared the sex ratios of these infants with respect to blastocyst quality, embryo growth rate and morphology.

Results

The main outcomes of this study were that the sex ratio at birth of single blastocyst frozen thawed embryo transfer babies was 56.67% which was higher than the normal level (51.22%). The sex ratio of mothers older than 40 years was significantly lower than that of mothers younger than 40 years (0.39 vs 1.3 , $P < 0.05$). Transplanting good quality blastocysts significantly increased the proportion of boys (1.35 vs 0.94 , $P < 0.05$). Transplanting grade 5 and 6 blastocysts significantly increased the proportion of male babies born compared with grade 3 and 4 blastocysts (1.91 vs 1.28 , $P < 0.05$). There were no significant differences in the sex ratio with respect to the inner cell mass (ICM) score. In addition, the higher the trophectoderm (TE) score, the higher the sex ratio (2.79 vs 1.18 vs 0.91 , $P < 0.05$).

Conclusions

Our study indicated that blastocyst quality, especially trophoblastic cell score, had a significant effect on sex ratio.

Introduction

Human sex ratio is an important indicator of the gender balance of men and women. It is influenced by many factors. The sex ratio at birth, also known as the secondary sex

ratio (SSR), can be defined as the proportion of males in all live births. The SSR in most gender-neutral countries is around 1.05[1], which is an ideal level for maintaining social stability and promoting economic development.

Over the past decades, with the rapid popularity of assisted reproductive technology (ART) for infertility treatment, a growing number of babies were born by ART[2]. Gametes and embryos were exposed to external factors during ART, which might have an effect on SSR compared to natural pregnancy.

Most studies had demonstrated that fewer male infants are born following ICSI than with natural pregnancy and insemination[3-5]. It has also been reported that blastocyst stage transplantation might

increase the SSR when it was compared with cleavage stage transplantation [4, 6-8]. Other researchers have also observed a trend of a higher SSR in babies born after thawed embryos transferred than fresh embryos transferred[9].

There had been lots of studies showing the SSRs brought by ART and exploring the influencing factors of the SSR. However, there are few studies on the effect of blastocyst quality on the sex ratio of ART babies. This study aimed to determine whether the differences in the blastocyst quality, embryo growth rate and morphology at our hospital affected the sex ratio of live births via in single blastocyst frozen thawed embryo transfer cycle.

Methods

Study design and participants

The data was collected from Center for Reproductive Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. This retrospective study reviewed 4205 singleton infants born following in single blastocyst frozen thawed embryo transfer cycle from January 2016 to October 2020. The study had been approved by the ethics committee of the The Third Affiliated Hospital of Guangzhou Medical University. And written informed consent was obtained from each participant.

ART procedures

Women were monitored and managed according to the hospital's clinical protocols. Various controlled ovarian stimulation (COS) protocols were used, with 150-450 IU/day of recombinant FSH or human menopausal gonadotropin in a gonadotropin-releasing hormone antagonist protocol, a long agonist protocol, or a short agonist protocol. The protocols were determined according to each patient's characteristics (age, body mass index (BMI), AFC and AMH). Transvaginal oocyte retrieval was scheduled 35-36 h after hCG injection ART was performed per standard operating procedure of the hospital. Fertilization was assessed by the appearance of two pronuclei. The embryos were frozen and thawed in accordance with the protocols of the Vitricification Kit (Kitazato Corporation, Shizuoka, Japan). The luteal phase was supported by vaginal administration of micronized progesterone (400 mg/d) started on the day of ovarian puncture.

Blastocyst assessment

Blastocysts were classified using Gardner's score according to blastocyst size, morphology of the inner cell mass (ICM) and trophectoderm (TE) [10]. High quality blastocyst definition: 4BB, 4BA, 4AB, 4AA, 5BB, 5BA, 5AB, 5AA, 6BB, 6BA, 6AB, 6AA. Definition of non-good blastocyst: 3BC, 3CB, 3BB, 3BA, 3AB, 3AA, 3AC, 3CA, 4BC, 4CB, 4CA, 4AC, 5BC, 5CB, 5CA, 5AC, 6BC, 6CB, 6CA.

Statistical analysis

SPSS software (Version 24.0, IBM SPSS, IBM Corp, Armonk, NY, USA) was used for analysis. The proportion of male live births is used in this study as the SSR. Data were stratified by categorical variables. Univariate logistic regression analyses were performed to examine the correlations of various variables with SSR. $P < 0.05$ was considered to be significant.

Results

A total of 4205 babies were included in this study. There were 2383 male babies and 1822 female babies. The total SSR of the sample was 56.67%. The SSRs of different quality blastocyst were different (see Table 1).

Univariate logistic regression analyses showed that paternal age, blastocyst assessment, embryo growth rate and TE score were associated with SSR ($P < 0.05$). There were no significant differences in the sex ratio with respect to the inner cell mass (ICM) score. In terms of paternal age, the babies from highest paternal age group had a lower SSR than that from youngest age group ($P < 0.05$). And for blastocyst assessment, the SSR was significantly lower in poor-quality blastocysts group than good group ($P < 0.05$). Transplanting grade 5 and 6 blastocysts significantly increased the proportion of male babies born compared with grade 3 and 4 blastocysts ($P < 0.05$). In addition, the higher the trophectoderm (TE) score, the higher the sex ratio ($P < 0.05$) (Table 2). We also demonstrated that after controlling for the inner cell mass, trophoblast grade A was positively correlated with the highest sex ratio among singleton births (Table 3).

Discussion

SSR is often used as an important indicator of population health and fertility. As far we know, the normal value of SSR is 51.22%[1]. Many population-based retrospective study confirms that a specific ART regimen can alter the SSR of babies born, the SSR of babies born through ART was a little higher [11-13]. In the past, the research mainly focused on the influence of parental age, fertilization methods, embryo morphology, types of embryos transferred. However, the effect of blastocyst quality on sex ratio has not been studied yet.

We have analyzed for the first time the effects of blastocyst quality on the gender of the children born in our center after single blastocyst frozen thawed embryo transfer. The main findings of this study were as follows: There were 2383 male babies and 1822 female babies. The SSR of ART babies in single blastocyst frozen thawed embryo transfer cycle was 56.67% (Table 1); SSR was associated with blastocyst quality. Comparing to high quality blastocyst, poor quality blastocyst might decrease the percentage of male babies. In particular, the higher the score of TE, the greater the proportion of male babies (Table 2-3).

Recent evidences from meta-analysis and large population based studies have suggested that blastocyst embryos transfer is associated with sex ratio imbalance towards males[14, 15]. And for types of embryos

transferred, the SSR was significantly lower in fresh embryo transfer group than thaw transfer group. However, the effect of blastocyst quality on sex ratio in single blastocyst frozen thawed embryo transfer cycle has not been studied yet. We analyzed human sex ratio at birth of different blastocyst quality in single blastocyst frozen thawed embryo transfer cycle.

Our results are similar to those from a previous population-based study [16]. Our data showed that maternal age was associated with SSR ($P < 0.10$). The babies from highest paternal age group had a lower SSR than that from youngest age group. Probably because women over 35 years old, the uterus and ovaries will release a large number of alkaline substances, which is not conducive to the survival of the Y chromosome.

In our research, blastocyst quality was also implicated with SSR. Those poor quality blastocyst has an imbalance towards lower SSR compared with good quality blastocyst. In particular, the effect of TE cells was more obvious. Unlike with TE score, no effect on SSR was shown when compared to ICM score. We note that male blastocysts may grow more rapidly and receive better morphological scores than female blastocysts. According to Thomas et al., the observed correlations of trophectoderm performance with infant sex and the rates of implantation, pregnancy and pregnancy loss indicate that this measure will eventually be prioritized over the inner cell mass score[17]. Interestingly, we also demonstrated that after controlling for the inner cell mass, trophoblast grade A was positively correlated with the highest sex ratio among singleton births. However, these variations clearly emphasize the tendency of male embryos to reach the final stages of blastocyst development more rapidly than female embryos[18].

This study also has some limitations. Firstly, we only get samples from one reproductive center, further expansion of samples in future analysis is needed to validate our current fundings. We also included only single-blastocyst FET cycles that resulted in singleton live births to enable us to control the within-transfer characteristics that are known to affect outcomes.

Declarations

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Authors' contributions

Conceptualization: Yuling Mao and Ya-Ming Meng; methodology: Chunyan Wang;

formal analysis and investigation: Ming Zeng; writing - original draft preparation: Jingda Qiao; writing - review and editing: Lei Li; funding acquisition: Lei Li and Ya-Ming Meng; The author(s) read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. Each patient signed informed consent on obtaining and analyzing their clinical data prior to the initiation of IVF/ICSI-ET treatment. Informed consent was obtained from all individual participants included in the study.

Consent for publication

The author confirms that the study described has not been published before, that its publication has been approved by all co-authors and that its publication has been approved (tacitly or explicitly) by the responsible authorities at the institution where the study was carried out.

Competing interests

The authors declare.

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Tables

Table1 SSRs of babies from different quality Blastocyst

Blastocyst assessment	Male babies	Female babies	Total births	SSR(%)
3AA	2	3	5	40.00
3AB	26	31	57	45.61
3AC	2	4	6	33.33
3BA	1	0	1	100.00
3BB	57	42	99	57.58
3BC	9	9	18	50.00
3CB	0	1	1	0.00
4AA	561	319	880	63.75
4AB	586	522	1108	52.89
4AC	18	25	43	41.86
4BA	216	129	345	62.61
4BB	628	523	1151	54.56
4BC	123	126	249	49.40
4CA	2	1	3	66.67
4CB	24	20	44	54.55
5AA	28	8	36	77.78
5AB	28	17	45	62.22
5AC	1	0	1	100.00
5BA	13	4	17	76.47
5BB	35	21	56	62.50
5BC	6	10	16	37.50
5CB	1	0	1	100.00
6AA	3	0	3	100.00
6BA	3	0	3	100.00
6BB	10	6	16	62.50
6BC	0	1	1	0.00
total	2383	1822	4205	56.67

Table 2 Univariate logistic regression analyses of different variables with SSR

Variables	Male babies	Female babies	Total births	SSR(%)	χ^2	<i>P</i>
Maternal age group					23.504	0.0001
20-29	952	731	1683	56.57		
30-39	1411	1044	2455	57.47		
40-49	19	49	68	27.94		
Expansion degree					11.278	0.001
good-quality blastocysts	2197	1625	3822	57.48		
poor-quality blastocysts	186	197	383	48.56		
embryo growth rate					6.701	0.01
Grade 3-4	2255	1755	4010	56.23		
Grade 5-6	128	67	195	65.64		
ICM score					1.173	0.556
A	1255	929	2184	57.46		
B	1101	871	1972	55.83		
C	27	22	49	55.10		
TE score					193.657	0.0001
A	1293	464	1757	73.59		
B	1395	1183	2578	54.11		
C	159	175	334	47.60		

Table 3 Multivariate logistic regression analyses of different variables with SSR

	TE score	Male babies	Female babies	Total births	SSR(%)	χ^2	<i>P</i>
ICM score A	A	594	330	924	64.29	32.828	<0.0001
	B	640	570	1210	52.89		
	C	21	29	50	42.00		
ICM score B	A	233	133	366	63.66	15.336	<0.0001
	B	730	592	1322	55.22		
	C	138	146	284	48.59		