

Sex ratio of infants born through *in vitro* fertilization and embryo transfer: Results of a single-institution study and literature review

Chinatsu Nagata¹, Keiko Mekaru¹, Keiya Gibo¹, Rie Nakamura¹, Sugiko Oishi¹, Maho Miyagi¹, Kozue Akamine¹, Yoichi Aoki¹

¹Department of Obstetrics and Gynecology, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan

ABSTRACT

Objective: The possible effects of Assisted Reproductive Technology (ART) on sex ratio at birth are extremely significant. This study aimed to determine whether ART affects the sex ratio of infants born through *in vitro* fertilization and embryo transfer (IVF-ET).

Materials and Methods: We ran this retrospective study on 290 singleton infants born following IVF-ET from February 2014 to August 2018 at a single institution. We compared the sex ratios of these infants with respect to insemination versus intracytoplasmic sperm injection (ICSI), early-cleavage embryo versus blastocyst transfer, fresh versus frozen-thawed embryo transfer and normal sperm versus asthenospermia.

Results: There were no significant differences in the sex ratio with respect to the fertilization method, transfer time, fresh embryo or frozen-thawed embryo transfer. In addition, the multiple logistic regression analysis revealed that these factors did not significantly affect the sex ratio.

Conclusions: Our study indicated that the differences in the fertilization method transfer time and sperm motility rate did not affect the sex ratio of IVF live births. However, with increasing numbers of ICSI and blastocyst transfer cycles, factors possibly affecting the sex ratio need to be further investigated.

Keywords: Fertility and assisted reproduction, Sex ratio, Intracytoplasmic sperm injection, Implantation, In vitro fertilization and embryo transfer, Insemination

INTRODUCTION

The Japanese Society of Obstetrics and Gynecology's ART registry system reported the total number of ART treatment cycles in 2017 to be 448,210, resulting in 56,617 live births (Ishihara *et al.* 2020). In Japan, 1 in every 16.7 infants is conceived through ART. Both the number of treatment cycles and the number of infants born through ART have been increasing. In 2017, 4,826 live births involved ICSI, and the cumulative number of live births involving ICSI was 112,620.

According to the 2017 Comprehensive Report of Vital Statistics released by the Ministry of Health, Labor and Welfare (2018), the total number of live births in Japan was 946,065. The distribution by sex was 484,449 males and 461,616 females, for a sex ratio at birth (the ratio of males to females) of 1.05. There are multiple reports that fewer male infants are born following ICSI than with natural pregnancy and insemination (Maalouf *et al.*, 2014; Hentemann *et al.* 2009; Dean *et al.* 2010; Tarin *et al.* 2014; Arikawa *et al.* 2016). It has also been reported that the male birth rate increases if a blastocyst transfer is

performed (Maalouf *et al.*, 2017; Dean *et al.* 2010; Tarin *et al.* 2014). Therefore, the fertilization method and the stage at transfer may affect the sex ratio. Moreover, as a technician selects the sperm used in ICSI, concerns have been raised over the effects of human intervention on the sex ratio. If the use of ART continues to increase, the sex ratio may be affected.

This study aimed to determine whether the differences in the fertilization method, time of transfer, sperm motility rate or different ICSI technicians at our hospital affected the sex ratio of live births *via in vitro* fertilization.

MATERIALS AND METHODS

Study design and participants

This retrospective study reviewed 290 singleton infants born following *in vitro* fertilization and embryo transfer (IVF-ET) from February 2014 to August 2018 at the University of the Ryukyus Hospital (Okinawa, Japan). This study was carried out according to the guidelines of the Declaration of Helsinki. The ethical review board of the University of Ryukyus (Okinawa, Japan) approved the study protocol, and all patients provided informed consent prior to their inclusion in the study.

IVF protocol

In preparation for IVF/ICSI, we used either a long or short gonadotropin-releasing hormone agonist protocol or a gonadotropin-releasing hormone antagonist protocol for controlled ovarian stimulation. Protocol selection was based on the patient's ovarian function. Oocyte pick-up was conducted when the dominant follicles were ≥ 18 mm in diameter using transvaginal ultrasound. Fertilization was performed using IVF or ICSI. We collected the sperm *via* the swim-up method.

The embryos were cultured at 37.0°C using a supply gas concentration of 5.0% CO₂, 5.0% O₂ and 90.0% N₂ in a water-jacketed or drawer-type incubator. Fertilization was confirmed after 18–20h. Normal fertilization was confirmed by observing a male or female pronucleus.

The embryos were evaluated using the Veeck's classification (Veeck, 1988) system for early-cleavage embryos and the Gardner's classification system (Gardner *et al.* 2000) for blastocysts. We defined asthenospermia as total sperm motility < 40% and normal sperm as total sperm motility > 40% in undiluted semen according to the World Health Organization (WHO) classification of subfertility (WHO, 2010).

The embryos were frozen and thawed in accordance with the protocols of the Vitrification Kit (Kitazato Corporation, Shizuoka, Japan) or the Cryotop Safety Kit (Kitazato Corporation, Shizuoka, Japan) manufacturer. All embryo transfers were performed using transabdominal ultrasound during either a hormone replacement cycle or a natural cycle.

Outcome measures

The sex ratios of the IVF products were compared with respect to insemination versus ICSI, early-cleavage versus blastocyst transfer, fresh versus frozen-thawed embryo transfer and normal sperm versus asthenospermia in undiluted semen. Additionally, the sex ratio of live births involving ICSI was determined for each embryologist (A or B).

Statistics

Either the χ^2 test or logistic regression analysis was applied for statistical purposes. A p -value < 0.05 was considered statistically significant. We used the JMP software (SAS Institute, Inc., Cary, NC, USA) for the statistical analyses.

RESULTS

The mean maternal age of the subjects was 35.4 ± 4.0 years. We had 290 singleton infants born following IVF-ET at our hospital between February 2014 and August 2018. Of these, 136 (46.9%) live-born infants were conceived using insemination, whereas 154 (53.1%) were conceived using ICSI. In addition, 76 (26.2%) live-born infants were conceived *via* early embryo transfer, whereas 214 (73.8%) were conceived *via* blastocyst transfer. Forty-five (15.5%) live-born infants were conceived *via* fresh embryo transfer, whereas 245 (84.5%) were conceived *via* frozen-thawed embryo transfer.

Table 1 presents the sex ratio (XY/XX) of the infants with respect to the fertilization method, transfer time and fresh versus frozen-thawed embryo transfer. There were no significant difference in sex ratio with respect to the fertilization method, transfer time or fresh versus frozen-thawed embryo transfer.

Table 2 presents the sex ratio with regard to differences in pre-treatment sperm motility rate, and each ICSI technician. There were no significant differences in the sex ratio about sperm motility rate or between individual technicians. We ran a logistic regression analysis for the data regarding the fertilization method, transfer time,

fresh versus frozen-thawed embryos, maternal age, and number of good-quality embryos transferred or sperm motility rate. None of these factors affected the sex ratio.

DISCUSSION

Table 3 depicts a review of a number of previous studies reporting the sex ratio of infants born by assisted-conception techniques. Although it has been reported that fewer male live-born infants are conceived *via* ICSI than through natural pregnancy and insemination (Maalouf *et al.*, 2017; Hentemann *et al.*, 2009; Dean *et al.* 2010; Tarin *et al.* 2014; Arikawa *et al.* 2016; Luke *et al.* 2009) we did not see significant differences in the sex ratio with regards to the fertilization method in our study population. In this study, blastocyst transfer cycles were performed after ICSI three times more frequently than early embryo transfer cycles, 75.3% (116/154 cycles) versus 24.7% (38/154 cycles), respectively. It has been reported that there were higher male infant birth rates following blastocyst transfer cycles than early embryo transfer cycles (Maalouf *et al.*, 2014), suggesting the lack of a significant difference in sex ratio between the two transfer techniques in this study. The fact that this blastocyst transfer cycle is frequently performed is considered a possibility that there is no significant difference in sex ratio in our study population.

Several reports have investigated the factors associated with a higher male infant birth rate found in blastocyst transfer cycles (Maalouf *et al.* 2014; Hentemann *et al.*, 2009; Dean *et al.* 2010). Genes controlling glucose uptake, metabolism and antioxidant enzymes are located on the X chromosome (Gutiérrez-Adán *et al.* 2000; Pérez-Crespo *et al.*, 2005). A study examining cow and mice embryos reported that the expression of gene coding for the enzymes glucose-6-phosphate dehydrogenase (G6PD) and hypoxanthine phosphoribosyl transferase, both involved in energy metabolism, is higher in female than in male embryos (Gutiérrez-Adán *et al.* 2000; Pérez-Crespo *et al.*, 2005). Human embryos have similar gene expression patterns. Although female embryos have higher energy metabolism, they may also be under oxidative stress

Table 1. Sex ratio (XY/XX) by fertilization method, transfer time and fresh or frozen-thawed embryo transfer.

Method of fertilization	Transfer time Fresh/frozen-thawed embryo transfer	Sex ratio (XY/XX)	p
IVF (n = 136)	Early embryo (n = 38) Blastocyst (n = 98)	1.53 (23/15) 1.13 (52/46)	0.43
	Fresh embryo (n = 30) Frozen-thawed embryo (n = 106)	1.31 (17/13) 1.21 (58/48)	0.85
ICSI (n = 154)	Early embryo (n = 38) Blastocyst (n = 116)	1.24 (21/17) 1.23 (64/52)	0.99
	Fresh embryo (n = 15) Frozen-thawed embryo (n = 139)	0.50 (5/10) 1.36 (80/59)	0.07

Table 2. Sex ratio by pre-treatment sperm motility rate and intracytoplasmic sperm injection (ICSI) technician.

Method of fertilization ICSI technician	Sperm motility ICSI technician	Sex ratio (XY/XX)	p
IVF (n = 136)	Normal sperm (n = 122) Asthenospermia (n = 13)	1.12 (65/58) 3.33 (10/3)	0.10
	Normal sperm (n = 92) Asthenospermia (n = 48)	1.14 (49/43) 1.40 (28/20)	0.57
ICSI technician	Technician A (n = 50) Technician B (n = 33)	1.08 (26/24) 2.00 (22/11)	0.19

Table 3. Review of reports of the sex ratio of offspring in assisted-conception pregnancies				
Author (year)	Country	Number of births	ART treatment	Sex ratio (% of male)
Hentemann <i>et al.</i> (2009)	Single institution (Norway)	420	ICSI Blastocyst (IVF)	0.69 1.17
Luke <i>et al.</i> (2009)	Society for Assisted Reproductive Technology National Database (USA)	15,164	Blastocyst (ICSI)	0.98
Dean <i>et al.</i> (2010)	Fertility clinics in Australia and New Zealand	13,368	IVF Blastocyst (IVF)	1.13 1.27
Maalouf <i>et al.</i> (2014)	United Kingdom	106,066	IVF ICSI Blastocyst (IVF+ICSI)	1.08 0.96 1.27
Arikawa <i>et al.</i> (2016)	Japan	27,158	IVF ICSI	1.13 0.92
Current study	Ryukyu University Hospital	290		No significant differences in sex ratio

due to this more active metabolism, making them more liable for growth retardation and poor quality than male embryos. This has been demonstrated by the fact that there is less growth retardation in female mice embryos cultured with G6PD inhibition (Pérez-Crespo *et al.*, 2005). However, it has been reported that in both human and cow embryos, glucose metabolism in the male embryo is significantly higher than that in the female embryo from the morula to blastocyst stages (Tiffin *et al.* 1991; Jiménez *et al.*, 2003). As such, male embryos may develop into blastocysts earlier than female embryos. Based on these findings, it has been suggested that male embryos develop into embryos that are more robust because they are less susceptible to oxidative stress. Moreover, male embryos develop into blastocysts earlier than female embryos because of the higher glucose metabolism in male embryos from the morula stage to the blastocyst stage. This means that more male blastocysts may be selected for fresh embryo transfer or during cryopreservation.

It has been reported that, in embryos fertilized with morphologically good sperm, more female embryos are selected for sperm injection. This is believed to be because sperm are selected under high magnification and that with a higher ratio of morphologically good and normal sperm, the percentage of sperm carrying an X chromosome is higher (Setti *et al.* 2012). Since ICSI involves artificial selection of good-quality sperm, it is possible that sperm carrying the X chromosome are preferentially selected, thus affecting the sex ratio.

Sperm motility rate may be associated with sex ratio during insemination. It has been reported that the male birth rate is significantly lower in groups with low-motility sperm (Arikawa *et al.* 2016). However, the male birth rate was lower from pre-treatment sperm motility rate for either insemination or ICSI at our hospital.

The culture environment also seems affect sex ratio. Compared with X-chromosome sperm, Y-chromosome sperm are more vulnerable to temperature changes and culture duration, and the expression of gene coding for proteins involved in apoptosis is higher in Y-chromosome sperm (You *et al.* 2017). In an experiment comparing blastocyst transfer in mice embryos cultured at 37°C or 41°C, the male to female sex ratio of the group cultured at 41°C was found to be lower than the ones cultured at 37 °C (Pérez-Crespo *et al.*, 2005). Because all of the embryos in our study were cultured in the same environment, culture environment was unlikely to have affected the sex ratio.

The possibility of sex selection in the endometrium was also considered, but another study failed to demonstrate sexual selection in mice endometrium, making this unlikely (Jiménez *et al.*, 2003). Therefore, the three factors that appear to affect sex ratio include the speed at which the embryo develops, the rate of embryo metabolism due to the sex of the embryo and embryo selection during transfer and cryopreservation.

CONCLUSION

Our study demonstrates that differences in the fertilization method, transfer time and sperm motility rate, and differences between individual ICSI technicians do not affect the sex ratio in IVF live births. However, with the increasing numbers of ICSI cycles and blastocyst transfer cycles being performed, additional factors that may affect the sex ratio need to be further investigated.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

Corresponding Author:

Keiko Mekaru
Department of Obstetrics and Gynecology
Graduate School of Medicine
University of the Ryukyus
Nishihara, Okinawa, Japan
E-mail: f041252@eve.u-ryukyu.ac.jp

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