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Original Article

ICSI preferentially influences female preterm birth

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Abstract: To determine the effect of IVF (*in vitro* fertilization) or ICSI (intracytoplasmic sperm injection) on the risk of preterm birth stratified by female and male? Patients seeking infertility treatment between January 2009 and December 2014 were included in this study. There were no difference in female and male BMI, pregnancy-related complications and congenital malformations. The average gestational age of singletons and twins in IVF group were significantly lower than the ICSI group (singleton: 38.5±1.8 vs. 38.6±1.7; P=0.008) (twin: 36.3±2.0 vs. 36.5±2.0; P=0.007). In the singleton cohort, IVF was associated with a higher risk of preterm births when compared with the ICSI group (8.6% vs. 7.1%; P=0.007). The same trend in the risk of preterm births was demonstrated in the twin cohort, IVF had also a higher risk of preterm births when compared with the ICSI group (42.3% vs. 37.8%; P=0.004). After adjustment for relevant confounding factors in a logistic regression model, ICSI yielded a lower risk of preterm singleton (aOR=0.80, 95% CI=0.67-0.96; P=0.018) and twin births (aOR=0.82, 95% CI=0.70-0.96; P=0.016). Finally, in the subgroup stratified by female and male, ICSI preferentially reduced the risk of female (aOR=0.756; 0.571-1.000; P=0.050) instead of male preterm singleton births (aOR=0.850; 0.666-1.085; P=0.192). In conclusion, ICSI might preferentially decrease female preterm singleton births.

Keywords: Preterm birth, singleton, intracytoplasmic sperm injection, *in vitro* fertilization

Introduction

The number of children conceived using assisted reproductive technology (ART) has exceeded 6 million since Louise Brown was born in 1978 (Adamson, ESHRE, Lisbon, 2015). IVF babies are at a higher risk of poor perinatal outcome compared with spontaneous conceptions. Of ART-conceived infants, 31% are low birthweight compared with 8.1% of all infants. Of ART infants, 5.7% are very low birthweight compared with 1.4% of all infants. Of ART infants, 36.2% and 6.7% are born preterm (PTB <37 weeks) and very preterm (VPTB <32 weeks) compared with 11.8% and 1.9% of all infants, respectively [1]. The proportion of ART babies ranges from 0.6%-5.9% of the total national birth population in 2010 according to the European Society of Human Reproduction and Embryology [2]. Hence, a better understanding of the causes of poor perinatal outcomes is crucial.

Plural gestations are associated with a high number of preterm births and low birthweight, and increase the risk of long-term health issues. Two recent studies from the United States [3] and Europe [2] showed that the proportion of multiple births was 26.5% and 20.6% among infants conceived from ART, respectively. Reducing the number of embryos transferred is an effective strategy to avoid poor perinatal outcomes associated with multiple births; however, evidence suggested that ART-conceived singleton pregnancies are also at high risk of low birth weight, preterm birth, small for gestational age (SGA), and perinatal mortality [4, 5]. "Vanishing twins" account for 7% of all singleton pregnancies; it has been reported that low birth weight, SGA, and preterm birth occur more frequently in singleton survivors of vanishing twins than singletons from initial singleton pregnancies [6, 7]. Elective single embryos are used to overcome the problems associated with multiple pregnancies. Nevertheless, a systematic review and meta-analysis have sug-

gested that singletons conceived from elective single embryo transfer are still at increased risk of preterm birth compared with spontaneous conceptions [8].

The causes of poor perinatal outcomes in ART infants are not completely clear. Different ART technology plays an important role in the risk of preterm birth. Previous study has suggested frozen-thawed embryo transfer could improve perinatal outcomes in relation to fresh embryo transfer [9]; blastocyst transfer has a higher risk of preterm births when compared with cleavage stage embryo transfer [10-12]. Moreover, it has been reported that ICSI is associated with lower preterm singleton births [13, 14]. The association of ICSI with lower risk of PTB may be due to sperm morphology screen in ICSI procedure. Previous study has suggested that a higher proportion of sperm with normal morphology seem to carry X chromosome [15]. Therefore, we hypothesize that ICSI may be preferentially associated with a low risk of female preterm birth in singletons cohort.

Materials and methods

Participants and stimulation protocol

This was a retrospective analysis of preterm births stratified by female and male, and included 10310 singletons and 4088 twins born from fresh embryo transfers between January 2009 and December 2014. All singletons and twins included in this study were the first children for couples. Women with previous reproductive history (multiparas) were excluded. Women underwent controlled ovarian hyperstimulation with a gonadotrophin-releasing hormone agonist or antagonist protocol. Ovarian follicle development was monitored based on serum estradiol levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm and the estradiol concentration was >500 pg/ml, human chorionic gonadotrophin (Serono, Aubonne, Switzerland) was administered before ultrasonography-guided oocyteretrieval. Luteal support was initiated on the day after oocyte retrieval using 60 mg of progesterone (Xianju Pharmacy, Zhejiang, China).

Laboratory protocol

IVF and ICSI were performed according to the routine laboratory insemination procedures on the day of oocyte retrieval. ICSI was used to treat male cause of infertility. Medical indications of ICSI included oligoasthenospermia, azoospermia, previous fertilization failure and unknown infertility etc. In the case of IVF, oocytes were inseminated in fertilization medium. After checking for the presence of two pronuclei and two polar bodies, zygotes were transferred to cleavage stage medium. In the case of ICSI, oocytes were cultured in cleavage stage medium after injection. After checking for the presence of two pro-nuclei and two polar bodies, zygotes were transferred to cleavage stage medium. Embryo morphology was evaluated 68-72 h after insemination with respect to cell number, size, and fragmentation. The number of embryos transferred was determined based on patient age, number of IVF cycles, and embryo quality. From April 2013, a maximum of two embryos were transferred into the uterus on day 3.

Clinical outcomes

Preterm birth was defined as birth at <37 weeks gestation. Very preterm birth was defined as birth <32 weeks gestation. Pregnancy complications, including pre-eclampsia, gestational diabetes, gestational hypertension, placenta previa, placenta abruption, placenta implantation, premature rupture of fetal membranes, vaginal bleeding, and severe anemia etc, were assessed. Congenital malformations included neonatal brain injury, congenital heart disease, Down syndrome, cleft lip and palate, congenital anal atresia, hypospadias, megacolon, Pulmonary dysplasia, and so on. In the twin cohort, at least one infant was defined as congenital malformations.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS, version 17.0; SPSS, Inc., Chicago, IL, USA). Continuous variables were compared using an independent sample *t*-test, and categorical variables were evaluated with a chi-square test. All tests were two-sided, and a p-value ≤ 0.05 was considered statistically significant. A logistic regression model was used to analyze the relationship between insemination method (ICSI vs. IVF) and preterm birth after adjusting for relevant confounding factors, including female and male age, female

Table 1. Patient and cycle characteristics and multivariate regression performed in 10310 singleton births

	IVF	ICSI	*P* value	β	aOR	95% CI for aOR	*p* value
Singletons (n)	5774	4536					
Female age (years)	32.2±4.0	31.3±4.3	<0.001	0.01	1.01	0.98-1.04	0.389
Male age (years)	33.8±4.9	33.3±5.5	<0.001	-0.01	0.99	0.97-1.01	0.360
Male BMI	25.2±3.6	25.3±3.7	0.461	0.01	1.01	0.99-1.03	0.259
Female BMI	22.3±3.3	22.4±3.3	0.871	0.00	1.04	1.02-1.07	<0.001
Male only factor	3.4% (199)	38.3% (1738)	<0.001		1.00		
Female only factor	68.4% (3949)	24.5% (1111)	<0.001	0.06	0.99	0.77-1.28	0.947
Female and male factor	25.1% (1452)	33.2% (1504)	<0.001	0.21	1.21	0.94-1.55	0.138
Unexplained factor	3.0% (174)	4.0% (183)	0.006	-0.07	0.92	0.56-1.53	0.758
Number of embryos transferred	2.1±0.4	2.1±0.5	0.315	0.02	1.02	0.70-1.48	0.929
Number of fetal sacs	1.1±0.4	1.1±0.4	0.144	0.19	1.21	0.88-1.66	0.252
Pregnancy-related complications	11.3% (670)	10.3% (491)	0.097	1.40	3.99	3.31-4.80	<0.001
Congenital malformation	1.9% (109)	1.8% (82)	0.824	1.65	5.26	3.74-7.41	<0.001
Gestational age	38.5±1.8	38.6±1.7	0.008				
Very preterm birth (<32 weeks)	1.0% (55)	0.8% (39)	0.599				
Preterm birth (<37 weeks)	8.6% (494)	7.1% (322)	0.007	-0.22	0.80	0.67-0.96	0.018

The adjusted outcome was the odds of preterm birth with ICSI in relation to IVF. Data are presented as numbers (%) or mean ± SD.

Table 2. Patient and cycle characteristics and multivariate regression performed in 4088 twin births

	IVF	ICSI	*P* value	β	aOR	95% CI for aOR	*p* value
Twins (n)	2273	1815					
Female age (years)	31.0±3.7	30.3±4.0	<0.001	0.03	1.03	1.00-1.05	0.042
Male age (years)	32.7±4.6	32.2±5.1	0.001	0.01	1.01	0.99-1.03	0.503
Female BMI	22.3±3.2	22.4±3.3	0.403	0.00	0.99	0.97-1.01	0.307
Male BMI	25.3±3.7	25.3±3.9	0.902	-0.02	0.98	0.96-1.00	0.043
Male only factor	3.3% (74)	39.7% (720)	<0.001		1.00		
Female only factor	68.4% (1555)	23.7% (431)	<0.001	-0.10	0.92	0.74-1.14	0.450
Female and male factor	24.8% (564)	31.5% (571)	<0.001	0.13	1.16	0.94-1.43	0.167
Unexplained factor	3.5% (80)	5.1% (93)	0.012	-0.04	0.97	0.66-1.44	0.887
Number of embryos transferred	2.2±0.4	2.2±0.4	0.018				
Number of fetal sacs	2.0±0.3	2.0±0.3	0.308				
Pregnancy-related complications	11.4% (271)	10.0% (193)	0.187	1.26	3.61	2.88-4.51	<0.001
Congenital malformation	5.1% (116)	4.9% (89)	0.829	0.71	2.08	1.52-2.84	<0.001
Gestational age	36.3±2.0	36.5±2.0	0.007				
Very preterm birth (<32 weeks)	3.3% (75)	3.1% (57)	0.790				
Preterm birth (<37 weeks)	42.3% (961)	37.8% (686)	0.004	-0.20	0.82	0.70-0.96	0.016

The adjusted outcome was the odds of preterm birth with ICSI in relation to IVF. Data are presented as numbers (%) or mean ± SD.

and male BMI, the period of embryo culture, the number of embryos transferred, the number of early gestational sacs, singleton versus multiple pregnancies, pregnancy-related complications, congenital malformations, and causes of infertility. In the logistic regression, dependent variable was preterm birth, independent variables were insemination method, female and male age, female and male BMI, number of fetal sac, number of embryo transferred, the

Table 3. ICSI preferentially decreases female preterm in singleton births

Singletons (n=10310)	IVF	ICSI	*P* value	AOR (95% CI)
Male (n=5470)				
Preterm birth (%)	8.80%	8.00%	0.279	0.850 (0.666-1.085)
Female (n=4840)				
Preterm birth (%)	8.20%	6.20%	0.008	0.756 (0.571-1.000)

AOR: adjusted by female/male age, female/male BMI, causes of infertility, pregnancy complication and congenital conformation.

day of embryo transfer, number of viable fetus, pregnancy complications, congenital malformations, and causes of subfertility. In independent variable, IVF, one fetal sac, one embryo transferred, D3 transfer, one viable fetus, no pregnancy complications, no congenital malformations, and male factor of subfertility cohorts were defined as reference group. The aOR indicated the odds of preterm birth with ICSI in relation to IVF.

Results

A total of 10310 singletons and 4088 twins were included in this retrospective study. In the singleton cohort, 5774 infants were born following IVF and 4536 infants were born following ICSI. In the twin cohort, 2273 twins were born following IVF and 1815 twins were born following ICSI.

In the singleton cohort, female and male age in the IVF group were greater than the ICSI group (female: 32.2±4.0 vs. 31.3±4.3 years, P<0.001; male: 33.8±4.9 vs. 33.3±5.5 years, P<0.001). No statistical significance was shown with respect to female and male BMI between the IVF and ICSI groups (female: 22.3±3.3 vs. 22.4±3.3 kg/m^2, P=0.871; male: 25.2±3.6 vs. 25.3±3.7 kg/m^2, P=0.461). The IVF group had a higher proportion of female cause of infertility (68.4% vs. 24.5%; P<0.001) and lower proportion of male cause of infertility (3.4% vs. 38.3%; P<0.001) in comparison with the ICSI group. The number of embryos transferred (2.1±0.4 vs. 2.1±0.5; P=0.315) and fetal sacs (1.1±0.4 vs. 1.1±0.4; P=0.144) in the IVF group was not different than the ICSI group. The proportion of pregnancy-related complications (11.3% vs. 10.3%; P=0.097) and congenital malformations (1.9% vs. 1.8%; P=0.824) were comparable between the IVF and ICSI group. The average gestational age in the IVF cohort was significantly lower than the ICSI cohort (38.5±1.8 vs. 38.6±1.7; P=0.008). Finally, the IVF group had a higher risk of preterm and very preterm births when compared with the ICSI group (PTB: 8.6% vs. 7.1%; P=0.007; VPTB: 1.0% vs. 0.8%; P=0.599 **Table 1**), however, the difference did not reach statistical significance with respect to very preterm birth. The same trend in patient and cycle characteristics was demonstrated in the twin cohort except for the number of embryos transferred. In agreement with the findings in the singleton cohort, IVF was also associated with a higher risk of preterm births (42.3% vs. 37.8%; P=0.004; **Table 2**) and very preterm births (3.3% vs. 3.1%; P=0.790; **Table2**) in the twin cohort.

To rule out the effect of confounding factors on the risk of preterm birth, a logistic regression model was used to adjust for female and male age, female and male BMI, the number of embryos transferred, the number of fetal sacs, period of embryo culture, singleton versus multiple pregnancies, pregnancy-related complications, congenital malformations, and causes of infertility. As shown in **Table 1**, the results indicated that oocyte insemination technology (ICSI vs. IVF [aOR=0.80, 95% CI=0.67-0.96; P=0.018]), female BMI (aOR=1.04, 95% CI=1.02-1.07; P<0.001), pregnancy complications (aOR=3.99, 95% CI=3.31-4.80; P<0.001) and congenital malformations (aOR=5.26, 95% CI=3.74-7.41; P<0.001) were associated with the risk of preterm singleton births. Similarly, as shown in **Table 2**, female and male age, female and male BMI, period of embryo culture, pregnancy-related complications, congenital malformations, and causes of infertility were adjusted with logistic regression. Oocyte insemination technology (ICSI vs. IVF [aOR=0.82, 95% CI=0.70-0.96; P=0.016]), female age (aOR=1.03, 95% CI=1.00-1.05; P=0.042), male BMI (aOR=0.98, 95% CI=0.96-1.00; P=0.043), pregnancy complications (aOR=3.61, 95% CI=2.88-4.51; P<0.001) and congenital malformations (aOR=2.08, 95% CI=1.52-2.84; P<0.001) were associated with the risk of preterm twin births. ICSI significantly reduced the risk of preterm singleton and twin births.

Most importantly, in the subgroup stratified by female and male, ICSI preferentially reduced

the risk of female (aOR=0.756; 0.571-1.000; P=0.050) instead of male preterm singleton births (aOR=0.850; 0.666-1.085; P=0.192), adjusted by female/male age, female /male BMI, causes of infertility, pregnancy complications and congenital malformations (**Table 3**).

Discussion

It is well-known that perinatal outcomes of IVF singletons are worse than singletons born following spontaneous conception. A Swedish study involving the youngest cohort born between 2002 and 2006 reported that the risk of preterm birth in IVF and ICSI singletons was still higher than non-IVF singletons [16]. Romundstad et al. suggested that the adverse outcomes in IVF singletons result from maternal-related factors [17]. In agreement with previous findings, a recent meta-analysis assessed the risk of preterm singleton births following spontaneous conceptions in subfertile women versus singletons born to fertile women; the pool estimated aOR for preterm births was 1.35 (95% CI=1.22-1.50) [18]. However, Henningsen et al. compared singleton siblings conceived from different ART technologies and the results indicated ART technology plays an important role in the risk of preterm birth, low birthweight, and mean birthweight [19]. For instance, several studies have shown a lower risk of preterm singleton births following ICSI [20-22]. The current study, for the first time, showed that the ICSI preferentially decreased female preterm birth in singleton cohort.

In the current study, the demographic and cycle characteristics in the IVF group, including female age, male age and female infertility factor, were greater than the ICSI group; female and male BMI, number of early gestational sacs, congenital malformations, and pregnancy-related complications in the IVF group were not different from the ICSI group. Pre-pregnancy BMI was associated with increased risk of preterm birth [23], pre-existing hypertension and pre-eclampsia or gestational hypertension with pre-eclampsia or anemia was significantly correlated with the risk of preterm birth [24]. Thus, female BMI and pregnancy-related complications as potential confounding factors were adjusted with logistic regression. In agreement with previous findings, our results showed that raised female BMI and pregnancy-related complications were associated with the risk of preterm singleton births. In addition, only cycles with fresh transfer were analyzed in this study because most studies reported a lower risk of preterm births in frozen-thawed transfers when compared with fresh transfer; a meta-analysis estimated that the aOR was 0.85 (95% CI=0.76-0.94, P=0.006) [18]. Moreover, blastocyst culture was associated with the risk of preterm birth; several studies reported that the risk of preterm birth among singletons was greater following blastocyst than cleavage transfer [10-12]. Period of embryo culture and the number of embryos transferred as potential confounding factors were adjusted with logistic regression. These two confounders were not associated with the risk of preterm singleton births, which was consistent with the previous findings [18, 25, 26].

The remarkable differences between the IVF and ICSI cohort were the causes of infertility, the ICSI group had a significantly lower proportion of female factor because ICSI was primarily used to treat male infertility. A lower risk of preterm births following ICSI may be related to a lower proportion of female factor. For this reason, we specifically analyzed the cause of infertility in the logistic regression model. Male only factor was used as reference, other causes of infertility were not associated with risk of preterm births in this logistic regression. The results indicated that ICSI was still associated with a lower risk of preterm births after adjustment for causes of infertility.

The unique finding of this study was that the ICSI preferentially reduced female preterm birth. Secondary, our study reported that ICSI also decreased the risk of preterm twin birth. Previously, other groups reported that ICSI had a significantly lower risk of preterm singleton births; however, a statistical difference was not detected in the twin cohort. Moreover, all studies on this issue did not report the effect of ICSI on preterm birth stratified by female and male. Indeed, male gender carried a higher risk of preterm birth than female [27, 28], and there was a gender bias between IVF and ICSI cohort [29, 30]. It was speculated that a lower risk of preterm birth following ICSI may be related to sperm morphology screen. Setti et al reported that a higher proportion of morphologically normal sperm under high magnification seem to carry X chromosome [15]. A higher proportion of sperm bearing-X chromosome might be

selected in ICSI procedure when compared with conventional IVF, in turn lead to more female offspring in ICSI cohort [29, 30]. Therefore, ICSI might preferentially decrease female preterm birth.

However, the limitations included an inability to adjust some additional confounding factors, such as a smoking habit. In addition, the previous reproductive history, including previous cesarean section (OR=2.904; 95% CI=1.066-7.910; P=0.037) or previous pre-term delivery (OR=3.412; 95% CI=1.342-8.676; P=0.010) is positively correlated with an increased risk of preterm birth [31]. We were not able to control these maternal factors because these confounding factors were not registered in our database; however, women with previous reproductive history were excluded from this study, as multiparas comprise only 5.7% of patients in our center.

In conclusion, the current study showed that ICSI might preferentially reduce the risk of female preterm birth in singleton with adjustment for relevant confounding factors. Further studies are needed to evaluate the safety of IVF laboratory technique, especially on the long-term health of ART infants.

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Disclosure of conflict of interest

None.

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