Congenital anomalies in infants conceived by infertile women through assisted reproductive technology: A cohort study 2004-2014

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Abstract. This retrospective cohort study aimed to analyse the risk of congenital anomalies (CAs) in infants conceived by infertile women through assisted reproductive technology (ART). A total of 9,013 clinical pregnancy cycles resulting in 9,101 live births between 2004 and 2014 were analysed. Congenital anomalies were evaluated and compared with spontaneous pregnancies in infertile women. A total of 9,101 infants were born following ART. Three subgroups were established: *In vitro* fertilisation fresh embryo transfer (IVF-ET), n=2,919, intracytoplasmic sperm injection fresh embryo transfer (ICSI), n=1,996 and frozen-thawed embryo transfer (FET), n=4,186. No significant differences in perinatal outcomes were observed between the three subgroups. A total of 105 (1.15%) infants were born with CAs. The birth defect rate was slightly higher in the IVF-ET subgroup compared with the other subgroups. Among infants in the IVF-ET and ICSI-ET subgroup, the probability of birth defects increased with increased maternal age (>35 years), male factors and diminished ovarian reserve. In the FET group, the risk of birth defects was significantly increased with multiple births and maternal age >35 years. The risk of congenital anomalies following ART was not significantly different compared with spontaneous conceptions within the infertile study population. The results of the present study may provide guidance for patients who are considering treatment for infertility in China.

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Introduction

The association between assisted reproductive technology (ART) and the risk of birth defects has previously been re reported (1-4). Patients undergoing conventional *in vitro* fertilization fresh-embryo transfer (IVF-ET) cycles often do not achieve fertilization (5). Total fertilization failure (TFF) increases the financial costs of an already stressful and expensive treatment plan and may result in further emotional strain on such patients (5). With frozen-thawed embryo transfer (FET), it is unclear whether the cryoprotectant, freezing or thawing procedures will have an adverse effect on the embryos and thereby increase the risk of major congenital anomalies (CAs) compared with ET procedures (6). Despite recent improvements in ART, it has been reported that TFF or near-TFF occurs in 15-20% of patients undergoing conventional IVF-ET cycles (7).

The number of children conceived through ART is now >5 million, and so determining whether there is an association between ART and birth defects is of great importance (8). It has previously been reported that maternal factors associated with infertility may in increase the risk of birth defects (9,10), or that an inherent defect responsible for infertility may also cause birth defects in the conceived child (11).

The aim of the present study was to retrospectively analyse ART data from 2004 to 2014 to determine whether CA rates are increased in infants conceived by infertile women via ART compared with those conceived by spontaneous conception (SC).

Materials and methods

Patients. The present study is a register-based cohort study. Women who underwent ART treatment, including IVF-ET, intracytoplasmic sperm injection fresh embryo transfer (ICSI) and FET, resulting in live birth between 2004 and 2014 were recruited from the Reproductive Medicine Centre of Tianjin Central Hospital of Obstetrics and Gynecology Tianjin, China). Inclusion criteria included patients exhibited a normal karyotype, normal levels endocrine hormone. Mean age of the patients was 31.56±4.34. All the recruited patients were contacted by telephone. If patients could not be reached by telephone, they were excluded from the study. A team of professional training

nurses assessed the course and the outcome of pregnancies via a telephone questionnaire and direct contact at one week post-partum. Direct consultations with obstetricians, paediatricians or ultrasound technicians were sought in all cases where an anomaly was suspected during conception. In total, 9,101 births after embryo transfer were assessed in the present study, including 4,186 births resulting from FET and 4,915 births resulting from IVF/ICSI-ET. All patients provided written informed consent and provided information regarding TFF and follow-up, including neonatal outcomes. The present study was approved by the Institutional Review Board of Tianjin Central Hospital of Obstetrics and Gynecology.

Clinical procedures and embryo transfer. All patients participating in this study underwent controlled ovarian stimulation according to routine long or short gonadotropin-releasing hormone (GnRH) agonist protocols (12). Pituitary suppression was achieved by daily subcutaneous injections of triptorelin acetate (100 μg; Ferring Pharmaceuticals Ltd., West Drayton, United Kingdom) initiated at the mid-luteal phase of the preceding cycle. The treating physician opted to use the GnRH agonist (3,978 patients) or antagonist (937 patients) protocol on the basis of patient characteristics or ovarian response during previous IVF cycles. The ovarian response during treatment was monitored by measuring serum E2 concentration and follicular growth (using vaginal ultrasound). Dosages of follicle-stimulating hormone and human menopausal gonadotropin were adjusted accordingly between 75 IU/day and 150 IU/day. Recombinant human chorionic gonadotropin (hCG; 5,000-10,000 IU/day; Lizhu Pharmacy, Zhuhai, China) was administered to trigger ovulation when two leading follicles reached a mean diameter of 18 mm measured by vaginal ultrasound. Oocyte retrieval was performed transvaginally 34-36 h following hCG administration. Oocytes were fertilized using IVF-ET or ICSI according to sperm quality. Sperm preparation, IVF-ET, ICSI and embryo culture were performed as previously described (7,12). Semen samples were collected by masturbation following 2-7 days of sexual abstinence. Samples were stored at 37˚C for 30 min for liquefaction, following which they were analysed for sperm count, motility and morphology according to World Health Organization criteria (13). During IVF-ET cycles, each oocyte was inseminated with 10,000 motile spermatozoa 3-4 h following retrieval. Patients whose partners were identified as having severe oligospermia or azoospermia in previous IVF-ET cycles received ICSI. During ICSI cycles, cumulus cells and the corona radiata of the oocytes were removed by exposure to HYASE (Vitrolife AB, Goteborg, Sweden) containing hyaluronidase for 10-15 sec at 2 h following retrieval. ICSI was performed on metaphase II oocytes as determined by observation under an inverted microscope (magnification, x200). The presence of two pronuclei was defined as normal fertilization and fertilized oocytes. All cells were grown in an incubator with a constant temperature of 37˚C and 5% CO_2, and continuously cultured in G1 medium (Vitrolife AB) for 2 days. All embryos from IVF-ET and ICSI were examined on the morning of day 3 following oocyte retrieval (~69 h after initial insemination). Every embryo was graded on the basis of the regularity of blastomeres and degree of DNA fragmentation: Grade 1, the sizes of the blastomeres were uniform, with no DNA fragmentation; grade 2, the blastomere sizes were slightly uneven with <20% DNA fragmentation; grade 3, the blastomere sizes were heterogeneous or DNA fragmentation was 20-50%; and grade 4, >50% DNA fragmentation. Good-quality embryos were defined as embryos with a grade of 1 or 2 (14). Only good quality embryos and ordinary quality embryos were selected for transfer (15). Typically, the two best-quality embryos were chosen for transfer on day 3, while surplus embryos of good or fair quality were cryopreserved or extensively cultured to the blastocyst stage for possible cryopreservation according to the protocol developed by Chinese legislation (16). From 2008 onwards, vitrification was used for embryo cryopreservation at this centre. Briefly, the embryos were equilibrated in equilibration medium [basal medium with 7.5% (v/v) ethylene glycol and 7.5% (v/v) dimethylsulphoxide (DMSO)] at room temperature for 10 min. The embryos were then transferred into the vitrification medium [basal medium with 15% (v/v) ethylene glycol, 15% (v/v) DMSO and 0.5 mol/l sucrose] at room temperature for 60 sec. The cryoprotectant-treated embryos were placed onto a fine Cryotop® (Kitazato BioPharma Co., Fuji, Japan) and then submerged immediately into liquid nitrogen ready for storage.

FET cycles were initiated during natural cycles following spontaneous ovulation as well as hormone replacement treatment (HRT) cycles. For the natural cycles, transvaginal ultrasound scans were performed on cycle days 10-12 to assess endometrial thickness, follicle growth and ovulation. FET was planned for 3 days after ovulation. Progesterone administration was initiated for luteal support 1 day after ovulation. For the HRT cycles, oral oestradiol (Progynova; Bayer AG, Leverkusen, Germany) was administered at a dosage of 2 mg/day on cycle days 1-4, 4 mg/day on cycle days 5-8 and 6 mg/day on cycle days 9-12. Transvaginal ultrasounds were performed to assess endometrial thickness and ovulation at day 13 and the oestradiol dosage was adjusted accordingly. When the endometrium reached ≥8 mm thickness, 40 mg of progesterone (Zhejiang Xianju Pharmaceutical Co., Ltd., Zhejiang, China) was administered intramuscularly. For the next 3 days, 60, 80 and 80 mg/day of progesterone was intramuscularly administered, respectively, as performed previously (17). Embryo transfer was performed on day 4.

Outcome measures. Serum human chorionic gonadotropin (hCG) was used to detect pregnancy 2 weeks after embryo transfer or 10 days after blastocyst transfer and was subsequently tested serially to monitor rising titres. Clinical pregnancy was defined as the presence of a gestational sac with foetal heart activity on ultrasound examination 5 weeks following oocyte retrieval. The implantation rate was defined as the number of gestational sacs per total number of transferred embryos (18). Neonatal outcome data were obtained by telephone interviews with parents following delivery. A questionnaire was used to obtain information of 9,013 clinical pregnancy cycles on gestational weeks, birth weight, sex and CAs. CAs were defined as all structural, functional and genetic anomalies diagnosed in aborted foetuses, at birth or during the neonatal period (19). CAs were classified and coded according to an extended version of the International Classification of Diseases (ICD-10) (20). Only cases with major CAs were included in the analysis and were categorized by organ system classification according to the ICD-10. Each

Table I. CA rates for live-born infants.

Parameter	Babies born with CAs (% of total in group)			χ^2	P-value
	IVF-ET	ICSI	FET		
Total	37 (1.27)	22 (1.10)	46 (1.10)	0.488	0.783
Sex					
Male	20 (1.27)	15 (1.51)	25 (1.14)	0.731	0.694
Female	17 (1.26)	7 (0.70)	21 (1.05)	1.804	0.406
Plurality					
Single	20 (1.20)	16 (1.34)	34 (1.58)	1.025	0.599
Multiple	17 (1.36)	6 (0.75)	12 (0.59)	5.502	0.064
Maternal age					
>35 years	13 (2.60)	4 (1.33)	15 (2.97)	2.182	0.336
<35 years	24 (0.99)	18 (1.06)	31 (0.84)	0.717	0.699

CA, congenital abnormality; IVF-ET, *in vitro* fertilization fresh embryo transfer; ICSI, intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer.

Table II. Types of congenital abnormalities among live-born infants.

ICD10 code	Total, n (%)	IVF-ET, n	ICSI, n	FET, n
Q00-Q07 central nervous system	7 (6.1)	4	1	2
Q10-Q18 eye, ear, face and neck	2 (1.7)	2	0	0
Q20-Q28 cardiovascular and circulation	46 (40.1)	17	9	20
Q30-Q34 respiratory	1 (0.8)	1	0	0
Q35-Q37 cheilopalatognathus	12 (10.2)	1	3	8
Q38-Q45 gastrointestinal	14 (12.1)	6	2	6
Q50-Q64 genitourinary system	7 (6.1)	3	1	3
Q65-Q79 musculoskeletal	11 (9.3)	5	3	3
Q80-Q89 other	4 (3.4)	0	2	2
Q90-Q99 chromosomal	13 (11.2)	1	3	9
Total no. birth defects[a]	114 (100.0)	39	24	51
Total no. of babies with birth defects	105 (92.1)	37	22	46

[a]Children with multiple defects appear more than once in the table. A total of 9 infants had multiple defects. ICD, International Classification of Diseases; ART, assisted reproductive technology; IVF-ET, *in vitro* fertilization fresh embryo transfer; ICSI, intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer.

organ system involved was recorded once per infant, however infants with multiple major anomalies may appear in several different groups depending on the affected organ systems. In addition, a concern with FET is often whether the cryoprotectants, freezing or thawing procedures have an adverse effect on the embryos and whether, thereby, cryopreservation processes increase the risk of major CAs (6). Therefore, only certain associated factors were analyzed during the FET process and certain characteristics of patients were not included.

Statistical analysis. Data were analysed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Parental reproductive and ART treatment parameters, maternal characteristics and pregnancy and birth outcomes in IVF-ET and FET groups were compared using Student's t tests for continuous variables and χ^2 tests for categorical variables. Statistical analysis using univariate logistic regression was used to evaluate the association between maternal age, infertility diagnosis, plurality and the risk of CAs in the FET group. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Pregnancy outcomes. The final sample population included 9,013 clinical pregnancy cycles resulting in 9,101 live births (IVF-ET, n=2,919; ICSI, n=1,996; FET, n=4,186). A total of 105 infants were born with CAs (Table I). In the ART subgroups, birth defects occurred in 37 infants conceived

Table III. Characteristics of maternal and treatment cycles of live birth in the IVF-ET group.

Parameter	SC (n)	CA (n)	P-value
Sex			0.275
Male	2,534	35	
Female	2,322	24	
Plurality			0.665
Single births	2,827	36	
Multiple births	2,029	23	
ART			0.601
IVF	2,882	37	
ICSI	1,974	22	
Maternal age (years)			0.009
>35	783	17	
≤35	4,073	42	
Abortion history			0.834
Yes	2,156	27	
No	2,700	32	
BMI			0.263
<23.0	2,732	36	
≥23.0-25.0	1,134	16	
≥25.0	990	7	
Infertility diagnosis			0.000
Male factor[a]	1,842	14	<0.001
Tubal factors	2,404	30	0.053
Diminished ovarian reserve	99	8	0.001
Uterine factors	23	1	0.385
Endometriosis	125	0	0.182
Ovulation disorders	87	3	0.428
Unexplained	246	3	0.487
Other factors	30	0	0.545
Duration of infertility (years)	4.16±3.04	4.56±3.33	0.32
Number of retrieved oocytes	14.05±7.58	13.37±7.46	0.496
Embryos transferred	2.10±0.55	2.15±0.15	0.443

[a]Male factor vs. Diminished ovarian reserve. SC, spontaneous conception; CA, congenital anomaly; IVF-ET, *in vitro* fertilization fresh embryo transfer; FET, frozen embryo transfer; BMI, Mass Index.

through IVF-ET (1.27%), 22 infants conceived through ICSI (1.10%) and 46 infants conceived through FET (1.10%). The birth defect rate was slightly higher in the IVF-ET subgroup compared with the other sub groups, however no significant difference was observed (Table I). For multiple births, the birth defect rate was slightly lower in the FET subgroup compared with the IVF-ET subgroup. For all subgroups, the birth defect rate in infants conceived by mothers aged >35 years was slightly but not significantly higher compared with those with mothers aged ≤35 years. The organ system distribution of birth defects is presented in Table II.

Parental factors. In the IVF-ET group, the number of birth defects was significantly higher with maternal age >35, male factor diagnoses and diminished ovarian reserve (P<0.05; Table III). In the FET group, an increased risk of birth defects was significantly associated with multiple births and maternal age >35 years (P<0.05; Table IV).

Multivariate analysis. Multivariate analysis was performed to determine independent predictors of CAs in the IVF-ET and FET groups. In the IVF-ET group, CAs were not significantly correlated with maternal age or infertility diagnosis (Table V). However, maternal age was an independent predictor of CAs in the FET group (P<0.05; Table IV).

Although there were fluctuations in clinical pregnancy rates during the study period, there was an overall increase in the clinical pregnancy rate in the IVF-ET group between 2004 and 2014 (Fig. 1). In the FET group, a substantial decline in the clinical pregnancy rate was observed from 2004 to 2005, followed by a period of no obvious change over the next 9 years (Fig. 1). Similarly, no significant differences in temporal trends

Table IV. Characteristics of maternal and treatment cycles of live birth in the FET group.

Parameter	SC (n)	CA (n)	P-value
Sex			0.769
Male	2,160	25	
Female	1,980	21	
Plurality			0.002
Single births	2,118	34	
Multiple births	2,020	12	
Maternal age (years)			0.000
>35	490	15	
≤35	3,650	31	
Embryo transfer	2.56±0.604	2.65±0.604	0.347

SC, spontaneous conception; CA, congenital anomaly.

Table V. Multivariate analysis to determine independent predictors of congenital anomalies in the IVF-ET and FET groups.

Parameter	Odds ratio	95% confidence interval	P-value
IVF-ET group			
Maternal age	1.623	0.341-1.114	0.109
Infertility diagnosis	1.156	0.803-1.665	0.435
FET group			
Plurality	1.134	0.737-1.744	0.567
Maternal age	1.799	1.313-2.466	<0.001

IVF-ET, *in vitro* fertilization fresh embryo transfer; FET, frozen embryo transfer.

for live birth delivery rates were observed between the groups. The spontaneous abortion rate in the IVF-ET group increased throughout the study period, reaching nearly 50% in 2014. Conversely, the spontaneous abortion rate declined in the FET group from 2004-2006 until 2006 and then plateaued until 2014 (Fig. 1). Ectopic pregnancy rates in the IVF-ET group decreased throughout the study period, however they remained higher compared with the FET group (Fig. 1). The multiple gestation rate decreased gradually in the IVF-ET group, whereas In the FET group a decline occurred from 2005 to 2008 followed by an increase from 2009 to 2014 (Fig. 1). Throughout the study period, the overall malformation rate remained relatively stable in the IVF-ET and FET groups (Fig. 1). None of the identified trends were statistically significant.

Discussion

The aim of the present study was to evaluate the risk of CAs in infants conceived through ART treatments, including IVF-ET and FET, relative to infants born after SC in infertile women; additionally, the impact of IVF-ET and ICSI on the risk of CAs was evaluated. The rate of CAs in infants conceived through ART infants ranged from 1.10-1.20%. Yin *et al* (21) previously reported the rate of major CAs to be 2.22% in ART infants in China. A meta-analysis published by Wen *et al* (22) reviewed 46 studies including 124,468 infants conceived through ART and reported a pooled risk estimation of 1.37. Another recent paper analysed the outcomes for infants born after ART treatments (23). The current study demonstrated that the rate of CAs (3.75%) was higher compared with that reported by the European Surveillance of Congenital Anomalies (EUROCAT; 2.0%) (23). However, the rate of CAs in infants conceived via ART was not significantly different compared with those conceived via SC within the infertile population.

In the present study, the prevalence of major anomalies and the distribution of anomalies in infants conceived through ART was similar to the data reported by the Chinese Center for Disease Control and Prevention (CDC) (24). The most frequent anomalies were heart defects, followed by gastrointestinal anomalies and anomalies of cheilopalatognathus. The underlying mechanisms responsible for the association between ART and CAs are uncertain and warrant further research. An excess risk of CAs in infants conceived through ART is associated

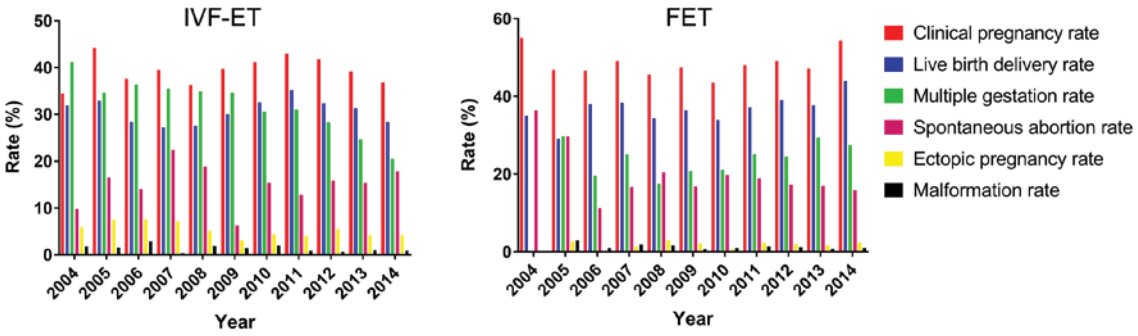

Figure 1. Pregnancy outcomes for patients undergoing IVF-ET or FET between 2004 and 2014. Clinical pregnancy rate refers to the number clinical pregnancies per 100 embryo transfer cycles. Live birth delivery rate refers to the number of deliveries that resulted in at least one live born baby per 100 embryo transfer cycles. Multiple gestation rate refers to the number of multiple gestation cycles per live birth delivery cycle. Spontaneous abortion rate refers to the number of spontaneous abortion cycles per live birth delivery cycle. Ectopic pregnancy rate refers to the number of ectopic pregnancy cycles per live birth delivery cycle. Malformation rate refers to the number of congenital anomalies per live birth. IVF-ET, *in vitro* fertilization fresh embryo transfer; FET, frozen embryo transfer.

with multiple factors (1). Factors associated with treatment that may increase the risk of birth defects include the age of infertile couples, underlying causes of infertility and medications used to induce ovulation or maintain the pregnancy in the early stages, which in turn may have effects on endometrial and cervical tissues and placentation or may impair embryo-endometrial synchronization. Furthermore, factors associated with ART procedures, including the culture media composition, length of culture, freezing and thawing of embryos, potential for polyspermic fertilization, delayed fertilization of the oocyte, altered hormonal environment at the time of implantation and manipulation of gametes and embryos may affect the risk of Cas (25,26). Older females (≥35 years) may explain the results of the current study as demonstrated by a previous study (27). Older females undergoing ART have an increased risk of producing abnormal gametes, resulting in poor obstetrical and perinatal outcomes (27). In the present study, maternal age parameters in the FET group were demonstrated to be correlated with CAs.

High rates of multiple births were observed in the present study. Previous studies have reported that ART leads to more multiple pregnancies compared with SC, the majority of which are twin pregnancies. Additionally, CAs are more frequent in twins compared with than single births (28-30). The results of the present study also suggest that the risk of CAs in infants conceived via ART may be associated with male factors. It has previously been reported that abnormal sperm morphology and subfertility in fathers is associated with hypospadias in offspring (31). The concern is that bypassing the natural protective barriers to poor quality sperm fertilization may be associated with an increased risk of future health problems in offspring (32). However, the risk of CAs in infants conceived via ICSI compared with those in the IVF-ET and FET groups was not significantly different.

The present study has a number of limitations. The most obvious is the reliance upon retrospective data, which may result in recall bias. Another disadvantage is that pregnancy outcomes in patients undergoing ART were not compared with naturally conceived infants at birth. Data were collected during the hospitalization at birth, and thus an evaluation of the delayed or long-term effects of ART was not attempted and would require extended follow-up. Although the present study was adjusted for maternal age uncontrolled or unmeasured risk factors could potentially produce biases.

To the best of our knowledge, this is the first study systematically designed to compare the risk of CAs with various ART methods. In summary, in the present study of 9,101 infants conceived via ART offspring, no significant increase in CAs was observed compared with those conceived via SC. This is consistent with a previous Chinese multicentre study (1.35%) (33). Although the majority of infants conceived via ART were free of birth defects, it is unclear whether other factors contributed to or could explain the observed associations. The results of the present study may be used to provide guidance when counselling patients who are considering treatment for infertility in China.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YH, HL and YZ contributed to project development and data collection. YH was responsible for writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Tianjin Central Hospital of Obstetrics and Gynecology. Informed consent was obtained from all participants included in this study.

Consent for publication

All data was anonymized, however written informed consent for publication of clinical data and clinical images was obtained from the patients.

Competing interests

All authors declare that they have no competing interests.

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