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ORIGINAL ARTICLE *Infertility*

No increased risk of major congenital anomalies or adverse pregnancy or neonatal outcomes following letrozole use in assisted reproductive technology

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STUDY QUESTION: Does letrozole use increase the risk of major congenital anomalies and adverse pregnancy and neonatal outcomes in fresh, single-embryo transfer?

SUMMARY ANSWER: Letrozole significantly decreases the risk of miscarriage and does not increase the risk of major congenital anomalies or adverse pregnancy or neonatal outcomes compared with natural cycles in patients undergoing ART.

WHAT IS KNOWN ALREADY: Letrozole is the most commonly used aromatase inhibitor for mild ovarian stimulation in ART. However, its safety in terms of pregnancy and neonatal outcomes is unclear.

STUDY DESIGN SIZE, DURATION: This retrospective cohort study used data from the Japanese national ART registry from 2011 to 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 3136 natural cycles and 792 letrozole-induced cycles associated with fresh, single-embryo transfer and resulting in a clinical pregnancy were included in the analysis. The main pregnancy outcomes were miscarriage, ectopic pregnancy and still birth, and the neonatal outcomes were preterm delivery, low birth weight, small/large for gestational age and major congenital anomalies. Terminated pregnancies were included in the analysis of major congenital anomalies. Odds ratios (ORs) and 95% CIs were calculated using multivariate logistic regression analysis adjusted for maternal age and calendar year.

MAIN RESULTS AND THE ROLE OF CHANCE: The risk of miscarriage was significantly lower in women administered letrozole (adjusted OR [aOR], 0.37, 95% CI, 0.30–0.47, $P < 0.001$). There was no significant difference in the overall risk of major congenital anomalies between the two groups (natural cycle 1.5% vs letrozole 1.9%, aOR, 1.24, 95% CI, 0.64–2.40, $P = 0.52$), and no increased risk for any specific organ system. Subgroup analysis demonstrated that the risk of major congenital anomalies was not increased in patients who underwent either in vitro fertilization or ICSI, or in those who received early cleavage stage or blastocyst embryo transfer. All other pregnancy and neonatal outcomes were comparable between the two groups.

LIMITATIONS REASONS FOR CAUTION: Despite the large sample size, we were only able to rule out the possibility that letrozole might cause large increases in birth-defect risks in ART patients.

WIDER IMPLICATIONS OF THE FINDINGS: The results suggest that letrozole stimulation reduces the risk of miscarriage, with no increase in the risk of major congenital anomalies or adverse pregnancy or neonatal outcomes compared with natural cycles in women undergoing ART. Letrozole may thus be a safe option for mild ovarian stimulation.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: Not applicable.

Key words: letrozole / congenital anomaly / pregnancy outcome / neonatal outcome / ovulation-induction method / mild ovarian stimulation

Introduction

Letrozole is the most commonly used aromatase inhibitor for mild ovarian stimulation in ART (Requena *et al.*, 2008; Kar, 2013). Letrozole blocks the synthesis of estrogen in ovarian granulosa cells, resulting in reduced levels of circulating and intraovarian estrogens and increased levels of intraovarian androgens (Velasco and Juan, 2012). Unlike clomiphene citrate, which is an alternative drug for mild ovarian stimulation, letrozole does not deplete estrogen receptors and maintains the normal central feedback systems thus facilitating normal follicular growth, selection of dominant follicles and ovulation (Velasco and Juan, 2012). These unique characteristics mean that letrozole has been the preferred ovulation-induction method for breast cancer patients undergoing ovarian stimulation to preserve fertility (Azim and Oktay, 2007; Goldrat *et al.*, 2015). It has also been suggested that letrozole may have a beneficial effect on the endometrium in terms of implantation, which may improve the success rate of embryo transfer (Miller *et al.*, 2012; Li *et al.*, 2014).

Despite its advantages, the safety of letrozole for infertility treatment remains controversial (Kar, 2013). Letrozole use was associated with increased risks of congenital cardiac and musculoskeletal abnormalities in neonates according to a conference presentation reported in 2005 (Biljan *et al.*, 2005; Novartis Pharmaceuticals Canada Inc., 2005; Tulandi *et al.*, 2006), following which its manufacturers, Novartis Pharma, issued a warning to physicians to stop using letrozole in non-menopausal patients. However, the conference presentation was never published in a peer-reviewed journal. Although several studies have subsequently evaluated the effects of letrozole on neonatal outcomes, including congenital anomalies, in non-ART populations (Mitwally *et al.*, 2005; Requena *et al.*, 2008; Kar, 2013; Diamond *et al.*, 2015), such studies remain scarce, and few results are available for patients undergoing ART (Requena *et al.*, 2008; Papanikolaou *et al.*, 2011; Kar, 2013). Moreover, pregnancy and neonatal outcomes following letrozole use in ART have been unclear because of its infrequent use due to the fear of potential congenital anomalies (Kar, 2013).

In this study, we evaluated the risk of major congenital anomalies, as well as pregnancy and neonatal outcomes, following letrozole use in patients undergoing ART, based on an analysis of a nationally representative sample in Japan.

Materials and Methods

Study design and sample selection

This was a retrospective cohort study using data collected between 2011 and 2013 by the Japanese national ART registry, established by the Japan Society of Obstetrics and Gynecology (JSOG). The data consisted of all cycle-specific information mandatorily reported by most ART clinics or hospitals in Japan (100% of 587 registered institutes in 2013; Saito *et al.*, 2015). The database contains information on the following: (i) patient age, infertility factors, ART procedure including ovulation-induction method, fertilization method, fresh or frozen status, embryo stage at transfer and luteal phase support; and (ii) pregnancy and neonatal outcomes including gestational week at delivery, birth weight, sex of neonate, mode of delivery and congenital anomalies. Information on congenital anomalies is usually obtained from ART clinics via reports from referral hospitals. To maintain a high follow-up rate, the JSOG strongly advised institutes to fill in any missing information and to contact the mothers directly if obstetric information could not be obtained from the delivery facility. The rate of known obstetric outcomes among pregnancy cases in 2013 was 97.7% (Saito *et al.*, 2015). The use of donor gametes or embryos is not allowed in Japan and all embryos were therefore autologous. Pregnancy termination is only allowed before 22 weeks of gestation in Japan, and all women undergoing terminations after 12 weeks of gestation must report the procedure to the governing body. Elective terminations performed before 22 weeks of gestation are usually performed in delivery facilities by specifically licensed doctors in Japan, and ART clinics then obtain delivery reports from the facility.

We included women who underwent natural or letrozole-induced cycles resulting in pregnancy after fresh-embryo transfer. We only included fresh cycles, to evaluate the effects of letrozole on both the embryo and endometrium. A natural cycle was defined as a cycle occurring without artificial ovulation induction, including a gonadotropin releasing hormone (GnRH) antagonist or agonist. Cycles induced using letrozole with additional human menopausal gonadotropin or recombinant follicle stimulation hormone or GnRH antagonist were excluded. We restricted the analysis to women undergoing single-embryo transfer, because multiple-transferred embryos might have been derived from different ovulation-induction cycles. Single-embryo transfer resulted in 51 twin pregnancies, including 46 (1.4%) from natural cycles and 5 (0.6%) from letrozole-induced cycles ($P = 0.066$). We excluded these twin pregnancies from our analysis because of the known association between multiple births and adverse neonatal outcomes. A flow diagram of the patient-selection process is shown in Fig. 1. Among 15 356 single-embryo transfer cycles, 11 262 cycles did not result in pregnancy (pregnancy rate for natural cycle, 24.9%; pregnancy rate with letrozole, 37.9%; $P < 0.001$). After excluding cases with missing or incomplete data on pregnancy outcome ($n = 115$) and twin pregnancies ($n = 51$), 3136 women with natural cycles and 792 with letrozole-induced cycles, with known pregnancy outcomes, were analyzed.

Ethical approval

All institutes had to obtain informed consent from the patients before use of letrozole for ovulation induction. This study was approved by the JSOG ethics committee and institutional review board at the National Center for Child Health and Development. The data were provided by the JSOG after approval, with no personally identifiable information.

Pregnancy and neonatal outcomes

Pregnancy outcomes included miscarriage before 22 weeks of gestation, ectopic pregnancy and still birth after 22 weeks of gestation. Neonatal outcomes included preterm delivery (PTD), very preterm delivery (VPTD), low birth weight (LBW), very low birth weight (VLBW), small for gestational age (SGA) and large for gestational age (LGA). PTD and VPTD were

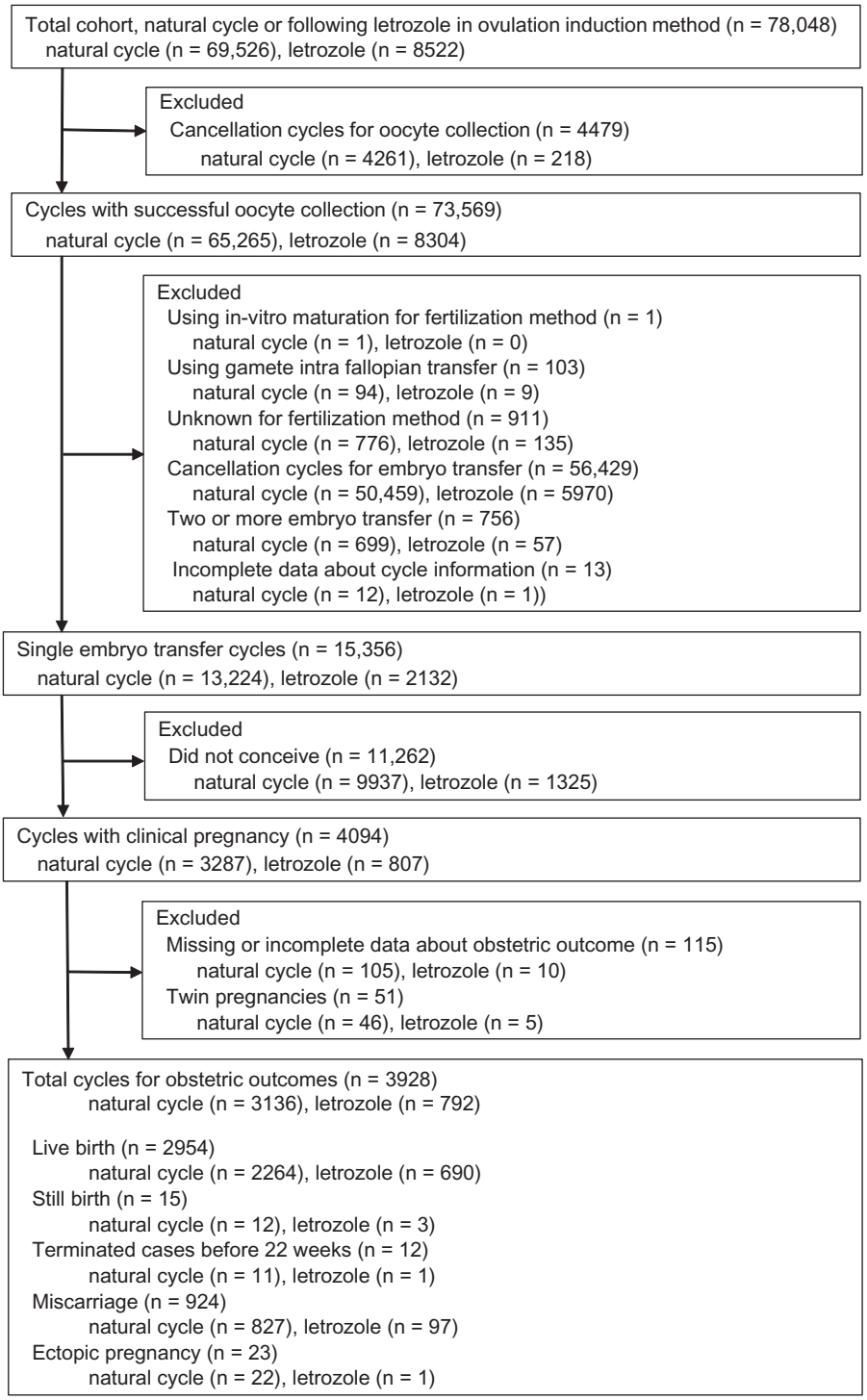

Figure 1 Flow diagram of cohort selection and comparison groups for ovulation-induction method.

defined as deliveries before 37 and 32 weeks of gestation, respectively. LBW and VLBW were defined as birth weights within 2500 g and 1500 g, respectively. SGA and LGA were defined as below the 10th percentile and above the 90th percentile, respectively, of the Japanese national reference for neonates born between 22 and 41 weeks of gestation (Itabashi *et al.*, 2010). We also analyzed sex of the neonate and mode of delivery as secondary outcomes.

Congenital anomalies

We included major and minor congenital anomalies identified before the end of the neonatal period, and excluded complications due to prematurity and suspected anomalies. Major congenital anomalies were defined according to the U.S. Centers for Disease Control and Prevention (CDC) guidelines (Rasmussen *et al.*, 2003). We classified all major congenital

anomalies according to organ system after blinded review of the abstraction forms by a board-certified obstetrician. To maintain the reliability of the classification, all cases were classified separately by the same person twice, and there were no discrepancies between the two classifications. Conditions that were not defined in the CDC guidelines were discussed by one board-certified obstetrician and one board-certified obstetrician/clinical geneticist, and some of these were adopted as major congenital anomalies, including acrania, duodenal atresia, procratresia, malrotation, polycystic kidney disease, congenital hydronephrosis, Klinefelter's syndrome and Turner's syndrome.

Statistical analysis

We compared the baseline characteristics, and pregnancy and neonatal outcomes following letrozole-induced and natural cycles using χ^2 or Student's *t*-tests. Continuous variables with non-normal distribution were analyzed using Mann–Whitney U tests. The trend for letrozole use between 2011 and 2013 was assessed by linear regression. Regarding neonatal outcomes, we restricted our analysis to cases with gestational ages between 22 and 45 weeks and birth weights between 200 g and 5000 g, which included all the samples. Regarding SGA and LGA, we excluded cases with missing information for gestational week at delivery, birth weight or sex of neonate (n = 77), and cases with gestational age >42 weeks (n = 16) from our analysis. In the analysis of congenital anomalies, we included live and still birth cases, as well as pregnancies terminated before 22 weeks of gestation. We calculated crude odds ratios (ORs) and 95% CIs for major congenital anomalies and pregnancy/neonatal outcomes following letrozole use by logistic regression. Adjusted ORs (aORs) were calculated including maternal age (categorized in 5-year intervals) and calendar year as confounding factors in the logistic model. For major congenital anomalies, we further conducted subgroup analyses stratified by fertilization method (IVF/ICSI) and embryo stage at transfer (early cleavage/blastocyst) to investigate if the effects of letrozole differed among different ART procedures. A two-tailed P value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Cancellation rates for oocyte collection and embryo transfer were significantly lower in letrozole-induced compared with natural cycles (2.6% vs 6.1% and 70.1% vs 72.6%, respectively, both $P < 0.001$). The pregnancy rates per embryo transfer (37.9% vs 24.9%, $P < 0.001$) and per registered cycle (9.5% vs 4.7%, $P < 0.001$) were significantly higher in letrozole-induced cycles compared with natural cycles (Fig. 1). The baseline characteristics of the cases stratified according to cycle type are shown in Table I. Mean maternal age was similar in both groups (natural cycle 36.3 years, letrozole-induced 36.2 years). Patients with a diagnosis of unexplained infertility were more likely to receive letrozole than patients with tubal factor, endometriosis or male factor infertility. Significantly more oocytes were retrieved after letrozole-induced cycles compared with natural cycles. Progesterone was more likely to be used for luteal phase support in letrozole cycles compared with natural cycles. Similarly, letrozole was used more frequently than natural cycles in ICSI and early cleavage stage embryo transfers. There was no trend in frequency for either type of cycle from 2011 to 2013.

Pregnancy and neonatal outcomes, including congenital anomalies, stratified according to cycle type are shown in Table II. Rates of miscarriage, ectopic pregnancy and pregnancy termination were lower in the letrozole group compared with the natural-cycle group, resulting in a higher live-birth rate. Among neonates, mean gestational week at delivery and birth weight were similar in both groups. The rates of preterm, LBW, SGA and LGA neonates were also similar in both groups. Major congenital anomalies were identified in 47 cases (1.6%) overall, including 34 cases (1.5%) in the natural-cycle group and 13 cases (1.9%) in the letrozole group. The clinical characteristics of the 13 cases with major congenital anomalies following letrozole use are shown in Supplementary data, Table SI.

The crude and aORs for letrozole-induced compared with natural cycles are shown in Table III. The risk of miscarriage was significantly lower

Table I Baseline characteristics of sample population stratified by ovulation-induction method (n = 3928).[a]

Characteristics	Natural cycle (n = 3136)	Letrozole (n = 792)	P value[b]
Maternal age (year)	36.3 (3.9)	36.2 (4.1)	0.187
<35	990 (31.6)	267 (33.7)	0.341
35–39	1467 (46.8)	348 (43.9)	
≥40	679 (21.7)	177 (22.3)	
Infertility diagnosis[c]			
Tubal factor	375 (12.0)	68 (8.6)	0.007
Endometriosis	140 (4.5)	16 (2.0)	0.002
Antisperm antibody	7 (0.22)	0 (0.0)	0.183
Male factor	495 (15.8)	92 (11.6)	0.003
Unexplained	2216 (70.7)	617 (77.9)	<0.001
Others	269 (8.6)	34 (4.3)	<0.001
Number of oocytes retrieved	1.2 (0.83)	1.7 (1.0)	<0.001
Fertilization method[d]			
IVF	1592 (50.8)	348 (43.9)	<0.001
ICSI	1488 (47.4)	399 (50.4)	
Split (IVF + ICSI)	56 (1.8)	45 (5.7)	
Embryo stage at transfer			
Early cleavage	2643 (84.3)	701 (88.5)	0.003
Blastocyst	493 (15.7)	91 (11.5)	
Luteal phase support			
None	274 (8.7)	77 (9.7)	<0.001
Progesterone	2437 (77.7)	678 (85.6)	
hCG	50 (1.6)	7 (0.9)	
hCG + progesterone	229 (7.3)	8 (1.0)	
Estrogen + progesterone	121 (3.9)	16 (2.0)	
Others	25 (0.8)	6 (0.8)	
Year[e]			
2011	802 (25.6)	68 (8.6)	0.502
2012	1202 (28.3)	408 (51.5)	
2013	1132 (36.1)	316 (39.9)	

[a]Data are presented as mean (SD) for continuous variables and n (%) for dichotomous variables.
[b]P values for all factors except year were assessed using χ^2 or Student's *t*-tests or Mann–Whitney U tests.
[c]Multiple answers were allowed.
[d]Denominators are numbers of fresh cycles for each ovulation-induction method.
[e]Percentages for rows in natural and letrozole cycles. P value was assessed by linear regression for trend.

Table II Pregnancy and neonatal outcomes stratified by ovulation-induction method.[a]

Characteristics	Total ($n = 3928$)	Natural cycle ($n = 3136$)	Letrozole ($n = 792$)	*P* value
Pregnancy outcomes				
Miscarriage	924 (23.5)	827 (26.4)	97 (12.2)	<0.001
Ectopic pregnancy	23 (0.58)	22 (0.70)	1 (0.13)	
Pregnancy terminated before 22 weeks of gestation	12 (0.31)	11 (0.35)	1 (0.12)	
Still birth after 22 weeks of gestation	15 (0.38)	12 (0.38)	3 (0.37)	
Live birth	2954 (75.2)	2264 (72.2)	690 (87.1)	
Neonatal outcomes[b]	($n = 2954$)	($n = 2264$)	($n = 690$)	
Gestational weeks at delivery (weeks)	38.7 (1.8)	38.7 (1.7)	38.6 (1.9)	0.139
<32	26 (0.88)	18 (0.79)	8 (1.2)	0.390
32–36	157 (5.3)	118 (5.2)	39 (5.7)	
≥37	2718 (92.1)	2083 (92.1)	635 (92.0)	
Unknown	53 (1.8)	45 (2.0)	8 (1.2)	
Birth weight (g)	3015 (439)	3022 (434)	2993 (454)	0.300
<1500	25 (0.85)	17 (0.75)	8 (1.2)	0.543
1500–2499	239 (8.1)	179 (7.9)	60 (8.7)	
≥2500	2646 (89.6)	2032 (89.8)	614 (89.0)	
Unknown	44 (1.5)	36 (1.6)	8 (1.2)	
Sex of neonates				
Male	1447 (49.5)	1116 (50.0)	331 (48.3)	0.527
Female	1458 (49.9)	1110 (49.7)	348 (50.7)	
Unknown	15 (0.51)	8 (0.35)	7 (1.0)	
Mode of delivery				
Vaginal	1959 (66.3)	1505 (66.5)	454 (65.8)	0.678
Cesarean section	888 (30.1)	674 (29.8)	214 (31.0)	
Unknown	107 (3.6)	85 (3.8)	22 (3.2)	
	($n = 2861$)	($n = 2187$)	($n = 674$)	
SGA[c]	232 (8.1)	175 (8.0)	57 (8.5)	0.705
LGA[c]	214 (7.4)	171 (7.8)	43 (6.4)	0.214
Congenital anomalies[d]	($n = 2981$)	($n = 2287$)	($n = 694$)	
Any major anomalies	47 (1.6)	34 (1.5)	13 (1.9)	0.869
Chromosomal abnormalities	15 (0.50)	11 (0.48)	4 (0.57)	0.756
Cardiovascular abnormalities	19 (0.64)	13 (0.56)	6 (0.86)	0.391
Musculoskeletal abnormalities	2 (0.067)	1 (0.043)	1 (0.14)	0.371
Any major or minor anomalies	59 (2.0)	44 (1.9)	15 (2.2)	0.694

[a]Data are presented as mean (SD) for continuous variables and *n* (%) for dichotomous variables.
[b]Denominators are number of live births excluding outliers for gestational week at delivery or birth weight ($n = 3$).
[c]SGA was defined as below the 10th percentile of the national reference. LGA was defined as above the 10th percentile of the national reference. Denominators are neonatal outcomes excluding those with unknown gestational week at delivery, birth weight, sex of neonate ($n = 77$) and those over 42 weeks of gestation ($n = 16$).
[d]Denominators are number of live births, still birth after 22 weeks of gestation and pregnancies terminated before 22 weeks of gestation.
SGA, small for gestational age; LGA, large for gestational age.

in the letrozole group (aOR, 0.37, 95% CI, 0.30–0.47). Similarly, the risk of ectopic pregnancy tended to be lower in the letrozole group, although the difference was not significant (aOR, 0.16, 95% CI, 0.02–1.23). The aORs for neonatal outcomes of PTD and LBW were 1.17 (95% CI, 0.82–1.66) and 1.16 (95% CI, 0.86–1.56), respectively, with no significant differences between the two groups. Similarly, the aORs for SGA and LGA were 1.03 (95% CI, 0.75–1.41) and 0.77 (95% CI, 0.54–1.10), respectively. The overall risks of major congenital anomalies were similar in both groups (aOR, 1.24, 95% CI, 0.64–2.40). There was no significant association between anomalies in any specific organ system and cycle type according to either crude or adjusted analysis. The ORs for all organ systems are shown in Supplementary data, Table SII.

The results of subgroup analyses of any major congenital anomalies stratified by fertilization method and embryo stage at transfer are

Table III Crude and adjusted ORs of letrozole use for pregnancy and neonatal outcomes compared with natural cycles.

Characteristics	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)[a]	*P* value
Pregnancy outcomes				
Miscarriage	0.39 (0.31–0.49)	<0.001	0.37 (0.30–0.47)	<0.001
Ectopic pregnancy	0.18 (0.02–1.33)	0.093	0.16 (0.02–1.23)	0.078
Still birth after 22 weeks of gestation	0.99 (0.28–3.52)	0.987	1.07 (0.30–3.83)	0.920
Neonatal outcomes				
Gestational weeks at delivery				
VPTD (<32 weeks)	1.45 (0.63–3.35)	0.383	1.37 (0.58–3.22)	0.478
PTD (<37 weeks)	1.13 (0.80–1.60)	0.474	1.17 (0.82–1.66)	0.397
Birth weight				
VLBW (<1500 g)	1.54 (0.66–3.59)	0.314	1.49 (0.63–3.57)	0.367
LBW (<2500 g)	1.15 (0.86–1.53)	0.351	1.16 (0.86–1.56)	0.333
Sex of neonates				
Male/Female	0.95 (0.80–1.12)	0.527	0.95 (0.80–1.14)	0.595
Mode of delivery				
Cesarean section	1.05 (0.87–1.27)	0.590	1.09 (0.90–1.31)	0.399
SGA	1.06 (0.78–1.45)	0.705	1.03 (0.75–1.41)	0.879
LGA	0.80 (0.57–1.14)	0.215	0.77 (0.54–1.10)	0.153
Congenital anomalies				
Any major anomalies	1.27 (0.66–2.41)	0.475	1.24 (0.64–2.40)	0.521
Chromosomal abnormalities	1.20 (0.38–3.78)	0.756	1.01 (0.32–3.21)	0.992
Cardiovascular abnormalities	1.53 (0.58–4.03)	0.394	1.53 (0.56–4.14)	0.405
Musculoskeletal abnormalities	3.23 (0.21–52.8)	0.399	2.82 (0.17–45.8)	0.465
Any major or minor anomalies	1.13 (0.62–2.03)	0.694	1.08 (0.59–1.98)	0.794

[a]Adjusted for maternal age and calendar year.
[b]SGA was defined as below the 10th percentile of the national reference. LGA was defined as above the 10th percentile of the national reference.
VPTD, very preterm delivery; PTD, preterm delivery; VLBW, very low birth weight; LBW, low birth weight; OR, odds ratio.

presented in Supplementary data, Table SIII. There were no significant differences in aORs according to IVF cycle vs ICSI cycle or early cleavage embryo transfer vs blastocyst transfer.

Discussion

The results of this study indicated that letrozole use was not associated with any significant increase in risk of major overall or organ-specific congenital anomalies compared with natural cycles in patients undergoing ART. Subgroup analyses demonstrated similarly non-significant effects of letrozole for different fertilization methods and different embryo stages at transfer. Pregnancy and neonatal outcomes tended to be better in the letrozole group, although the difference was only significant for risk of miscarriage, which was significantly lower in the letrozole group. These results indicate that the use of letrozole for ovulation induction in ART is safe and may reduce the risk of miscarriages in fresh embryo cycles. To the best of our knowledge, this is the first study to investigate the effects of letrozole on pregnancy and neonatal outcomes, including major congenital anomalies, using a large, nationally representative sample of patients undergoing ART.

Our results demonstrated that letrozole use was not associated with any increased risk of congenital anomalies compared with natural cycles. Although Novartis Pharma issued a warning against the use of letrozole in non-menopausal patients because of a potential risk of congenital anomalies (Biljan *et al.*, 2005), subsequent studies have demonstrated conflicting results (Tulandi *et al.*, 2006; Gill *et al.*, 2008; Sharma *et al.*, 2014). A recent randomized controlled study of letrozole for ovulation induction in 900 patients with unexplained infertility found no increased risk of major congenital anomalies (letrozole vs clomiphene citrate vs gonadotropin, 2/56 [3.6%] vs 3/70 [4.3%] vs 3/96 [3.1%], respectively, $P = 0.25$) (Diamond *et al.*, 2015). However, studies investigating the safety of letrozole in ART have often involved limited numbers of patients (10–87 individuals) (Requena *et al.*, 2008; Papanikolaou *et al.*, 2011; Kar, 2013; Eftekhar *et al.*, 2014).

We found that the miscarriage rate was significantly lower and the pregnancy and live-birth rates tended to be higher following letrozole-induced compared with natural cycles. Among 11 patients with unexplained infertility (5 with letrozole and 6 with natural cycles), letrozole use was associated with significantly increased integrin expression in the uterine endometrium compared with natural cycles (Ganesh *et al.*, 2014). Furthermore, letrozole increased integrin levels and significantly increased the pregnancy rate after IVF among women undergoing who lacked normal integrin expression (Miller *et al.*, 2012). Deficient integrin expression in the uterine endometrium was reportedly associated with low endometrial receptivity, which may result in implantation failure in infertile women (Lessey *et al.*, 1995; Casals *et al.*, 2010).

We therefore hypothesize that letrozole might increase integrin expression among ART patients with low integrin expression, thus improving miscarriage, pregnancy and live-birth rates compared with patients who do not receive letrozole (i.e. in a natural cycle). A similar low miscarriage rate was also reported in patients undergoing frozen-thawed embryo transfer cycles administered letrozole for endometrium preparation (Lee *et al.*, 2011; Hu *et al.*, 2014; Li *et al.*, 2014).

Our results demonstrated similar neonatal outcomes following letrozole-induced (n = 792) and natural cycles (n = 3136). Letrozole has a mean half-life of 45 h (range 30–60 h) and would thus be eliminated from the body by the time of implantation (Velasco and Juan, 2012; Palomba, 2015). To the best of our knowledge, no previous studies have investigated detailed neonatal outcomes in ART patients induced with letrozole (Requena *et al.*, 2008; Papanikolaou *et al.*, 2011; Kar, 2013; Eftekhar *et al.*, 2014). However, a randomized controlled trial in a non-ART population demonstrated that gestational week at delivery, birth weight, sex and neonatal complications were comparable in patients with letrozole and gonadotropin cycles (Diamond *et al.*, 2015), thus supporting the results of the current study.

This study had several important limitations. First, despite the large sample size, we were only able to rule out large increases in birth-defect risk. Power calculations indicated that the study had powers of 67.6% and 76.1% to detect relative risks of 2.0 for the outcomes of any major anomalies and any major or minor anomalies, respectively, and powers of only 10–32% to detect anomalies in specific organ systems and 14.9–64.6% to detect differences between different ART procedures. Based on our sample of 694 letrozole on-going pregnancies and 2287 natural-cycle on-going pregnancies, we could only detect birth-defect prevalence rates of ≤0.28% or ≥3.31% in the letrozole group with 80% power and an α-error of 5%. Further studies with larger sample sizes are therefore required to rule out the possibility of small risk differences, especially in terms of congenital anomalies. Second, the cancellation rates for embryo transfer and oocyte collection were high. The higher cancellation rates in natural cycles may have been the result of a spontaneous luteinizing hormone surge associated with oocyte development with relatively higher serum estradiol concentrations and smaller numbers of oocytes retrieved, respectively, compared with letrozole cycles. Third, information on the ovulation-induction regimen used by each clinic was not available in our registry. However four prior studies of letrozole use in Japan all used the same regimen (2.5 mg from cycle Day 3 or 5) (Shozu *et al.*, 2007; Shozu, 2007; Teramoto, 2012; Matsunaga and Ochi, 2013), and we believe that any differences in letrozole dose and administration schedules among the 40 clinics reporting data for this study would be minimal. Fourth, our study did not include a comparison group of naturally conceiving couples. However, a study including 10% of all Japanese births in 2009–2011 found a similar birth-defect-prevalence rate of 2.3% (Hirahara, 2013). Furthermore we only included major birth defects and had a shorter follow-up period compared with some previous studies (Reefhuis *et al.*, 2009; Fedder *et al.*, 2013; Pelkonen *et al.*, 2014). A small proportion of the birth-defect data in our study may also have been obtained through parental self-reports. Importantly however, the same birth-defect inclusion criteria and length of follow-up were used in both the natural- and letrozole-cycle groups in our study.

In conclusion, letrozole did not increase the risks of major congenital anomalies, or adverse pregnancy or neonatal outcomes, compared with natural cycles in patients undergoing ART. Furthermore, letrozole might reduce the risk of miscarriage in fresh-embryo cycles. Given that the need for mild ovarian stimulation is likely to increase, such as for the treatment of patients with low ovarian reserve and breast cancer patients preserving fertility, our results support the use of letrozole to achieve successful pregnancy in patients requiring ART. Despite the large sample size in the present study, further evidence is required to confirm the safety of letrozole use in ART.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

T.T. and S.C.J. conceived the study design. A.K. and H.S. collected data for the analysis. T.T. and S.C.J. analyzed the data, and T.T., S.C.J., A.K., M.I., T.K. and H.S. interpreted the results. T.T. wrote the first draft of the manuscript and S.C.J., A.K., M.I., T.K. and H.S. finalized the manuscript. All authors were involved in writing the paper and approved the final submitted versions.

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Conflict of interest

None declared.

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