

# Evaluation of birth outcomes, congenital anomalies and neonatal complications of singletons born to infertile women treated with letrozole: A retrospective cohort study

BIN WANG<sup>1</sup>, HONG LIN<sup>1</sup>, RUBIN XIA<sup>1</sup>, SHUIQIN LIN<sup>2</sup> and ZHILING LI<sup>1</sup>

<sup>1</sup>Reproductive Center, The First Affiliated Hospital of Shantou University Medical College, Shantou University, Shantou, Guangdong 515041, P.R. China; <sup>2</sup>Department of Pathology, The First Affiliated Hospital of Shantou University Medical College, Shantou University, Shantou, Guangdong 515041, P.R. China

Received December 24, 2023; Accepted May 17, 2024

DOI: 10.3892/etm.2024.12596

**Abstract.** At present, safety of letrozole administration as an ovulation-inducing drug still remains controversial. Investigation of the safety of letrozole use for the induction of ovulation in the Chinese population is scant. The present study aimed to fill this gap. Data concerning mothers using letrozole and birth outcomes of their singleton offspring were collected as the letrozole group (n=194), equivalent data from mothers using non-letrozole drugs and their singleton offspring were included as the non-letrozole group (control, n=154). Birth outcomes, congenital anomalies and neonatal complications were compared and analyzed between the two groups. Univariate analysis, Spearman's rank correlation analysis and the logistic regression model were utilized. For birth outcomes, the percentage of caesarean section deliveries in the letrozole group was lower than the non-letrozole group (43.8 vs. 56.4%,  $P=0.019$ ). For congenital anomalies, no significant difference was found between the two groups (all  $P>0.05$ ). The statistical  $P$ -value for the correlation between the maternal use of letrozole and neonatal complications

was marginal ( $P=0.051$ ). Results from the logistic regression analysis confirmed that maternal use of letrozole was not a significant contributor for neonatal complications, independent of statistical adjustment [crude odds ratio (OR), 1.436; 95% confidence interval (CI), 0.803-2.569;  $P=0.223$  vs. adjusted OR, 1.406; 95% CI, 0.748-2.643;  $P=0.290$ ). The results of the present study suggested that maternal use of letrozole for ovulation induction does not associate with poorer birth outcomes or increased risk of congenital anomalies and neonatal complications.

## Introduction

Letrozole, an aromatase inhibitor, is a commonly used drug for ovulation induction in assisted reproduction (1). It has several merits compared with clomiphene or human menopausal gonadotropin, including enhanced endometrial lining, improved cervical mucus, mono-follicular development and low risk of ovarian hyperstimulation syndrome (2-4). Moreover, letrozole is also widely accepted by sub-fertile patients owing to convenient oral administration and the low-cost of the treatment (5).

However, in 2005, Novartis, a major pharmaceutical producer of letrozole, issued a contraindication for letrozole use to physicians worldwide soon after the disputable abstract by Marinko Biljan in which higher risk of cardiac and locomotor anomalies were reported for neonates born from mothers using letrozole (6,7). In response to this controversy, a subsequent study indicated no association between letrozole use and increased risk of congenital anomalies (8). Despite all these findings, the restriction on letrozole as an ovulation-inducing drug has not been lifted yet.

Currently, in numerous developing countries such as China and India, letrozole, mainly used as an off-label drug, has gained great popularities among sub-fertile patients and reproductive physicians due to the aforementioned merits (9,10). Although previous studies have evaluated the impact of letrozole on neonatal birth outcomes, similar studies based on the Chinese cohort are inefficient (1,10-13). Such a study is necessary due to genetic heterogeneity, metabolic difference and discrepancy in protocol of ovulation induction. Furthermore,

---

*Correspondence to:* Professor Zhiling Li, Reproductive Center, The First Affiliated Hospital of Shantou University Medical College, Shantou University, 57 Changping Road, Jinping, Shantou, Guangdong 515041, P.R. China  
E-mail: stlizhiling@126.com

Mr. Shuiqin Lin, Department of Pathology, The First Affiliated Hospital of Shantou University Medical College, Shantou University, 57 Changping Road, Jinping, Shantou, Guangdong 515041, P.R. China  
E-mail: shuiqinlin@126.com

**Abbreviations:**  $\beta$ -hCG,  $\beta$ -human chorionic gonadotropin; CI, confidence interval; COS, controlled ovarian stimulation; IUI, intrauterine insemination; OR, odds ratio

**Key words:** birth outcome, congenital anomaly, letrozole, neonatal complication, ovulation induction

it may possibly provide us with more evidence concerning the safety of letrozole, and hopefully expedite the removal of restriction on letrozole use.

In the present study, birth outcomes, congenital anomalies and neonatal complications of singletons born to mothers who have received letrozole for ovulation were investigated. Neonates born to mothers who have either received other ovulation-inducing drugs or ovulated naturally were recruited as the control group.

## Materials and methods

**Study participants and design.** The electronic medical archives of women who had undergone natural cycles/ovulation-inducing cycles and intrauterine insemination (IUI) therapies in the Reproductive Center of The First Affiliated Hospital of Shantou University Medical College (Shantou, China) during January 1, 2016 and December 31, 2021 were retrospectively retrieved and analyzed. The inclusion criteria were as follows: i) Women who underwent natural or ovulation-inducing cycles resulting in pregnancies after IUI therapies; ii) women aged between 20 to 41 years, with a body mass index of  $<35 \text{ kg/m}^2$ ; and iii) delivered a live singleton. Further selection was performed according to the following exclusion criteria: i) Spousal sperm abnormalities; ii) endometriosis; iii) abnormalities of the uterus; iv) karyotypic abnormalities; and v) insufficient information. As illustrated in the flowchart of Fig. 1, a total of 348 women were finally enrolled, of which 194 women were defined as the study group (letrozole group; using letrozole to induce ovulation) and 154 women were defined as the control group (non-letrozole group; ovulating naturally or using non-letrozole ovulation-inducing drugs). Before the initiation of the therapy, each woman went through a set of standard fertility examinations in the center. Information about neonatal birth outcomes were collected via telephone interviews by trained senior nurses. Ethics approval was obtained from the Institutional Ethics Committee of The First Affiliated Hospital of Shantou University Medical College (approval no. 2015; Shantou, China). At the early stage (such as the recruitment stage) of the present study, all participants provided written informed consent. The written informed consent enabled the authors to collect data from the participants. All data collected were treated with confidentiality and anonymity. At the late stage of the present study, in order to acquire discharge summary for data validation from participants who did not deliver the infant in this hospital, additional verbal informed consent was obtained from the participants after adequate telephone communication (refer to subsection 'Independent validation of the data' for more information). Principles of The Declaration of Helsinki (the ninth revision, October 2013) were strictly adhered throughout the present study.

**Fertility examinations prior to therapy.** The fertility examinations were as follows: i) Visual inspection of the female reproductive organs; ii) Serological determination of sex hormones, including estradiol, progesterone, prolactin, testosterone, luteinizing hormone and follicle-stimulating hormone; iii) Hysterosalpingography for tubal patency test;

iv) Gynecologic ultrasonography to detect abnormalities of the uterus and adnexa, and, in particular, to assess ovarian function through evaluation of the morphology and size of the ovaries and calculation of the antral follicle count; v) Measurement of anti-Müllerian hormone when necessary. vi) Cervical cytology; vii) TORCH test, including toxoplasma, rubella, cytomegalovirus and herpes; viii) Screening for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), syphilis, chlamydia and gonococcus and ix) karyotype analysis when necessary. Items ii), v), vi), vii), viii) and ix) were completed in the Department of Clinical Laboratory of The First Affiliated Hospital of Shantou University Medical College. The rest of the examinations were completed in the Reproductive Center of The First Affiliated Hospital of Shantou University Medical College.

**The inclusion and exclusion criteria for vetting patients for IUI therapy.** The inclusion and exclusion criteria were as follows: i) Patients with vaginal atresia or complete transverse vaginal septum were excluded from IUI. Patients with congenital absence of uterus, rudimentary uterus, infantile uterus, hydrosalpinx, endometrial polyp, ovarian neoplasm, ovarian mass and chocolate cyst of ovary were excluded. Patients with cervical precancerous lesion or cervical carcinoma were excluded. ii) Patients with unilateral or bilateral tubal patency were eligible, those with bilateral tubal obstruction were excluded. iii) Patients with reproductive endocrinal disorder were excluded and advised to receive additional treatments. iv) Patients with adequate ovarian function were eligible, those with poor ovarian function were excluded and advised to receive *in vitro* fertilization. v) Patients with infertility caused by cervical factors were eligible; patients with immune infertility or unexplained infertility were eligible. vi) Patients with karyotypic abnormality or familial hereditary disease were excluded. vii) Patients with acute infection of the genitourinary system or sexually transmitted disease were excluded. Patients in acute stage of infection of any of the following pathogens were excluded: Toxoplasma, rubella, cytomegalovirus, herpes, HIV, HBV, HCV and HPV. viii) Patients with mental disorder were excluded.

**Protocols for controlled ovulation stimulation (COS) and IUI.** For each patient, the initiating dose of COS was individually tailored by a fertility physician according to age, body mass index, history of previous ovarian response and the present ovarian reserve. Generally, COS started 3-5 days after the menstruation. COS and IUI were then performed as described in a previous study by the authors (14). A total of 2 weeks after the IUI therapy, serum test for beta-human chorionic gonadotropin ( $\beta$ -hCG) was carried out to detect the existence of pregnancy. Luteal support was conducted as stated in a previous study by the authors (15). Live birth was defined as the birth of a live infant of  $>28$  weeks of gestation.

**Follow-up interview.** Follow-up interview was initiated two weeks after the IUI therapy and was carried out once every two months after that. With the help of a customized questionnaire (Table SI), a trained senior nurse collected data concerning maternal health, pregnancy and neonatal birth

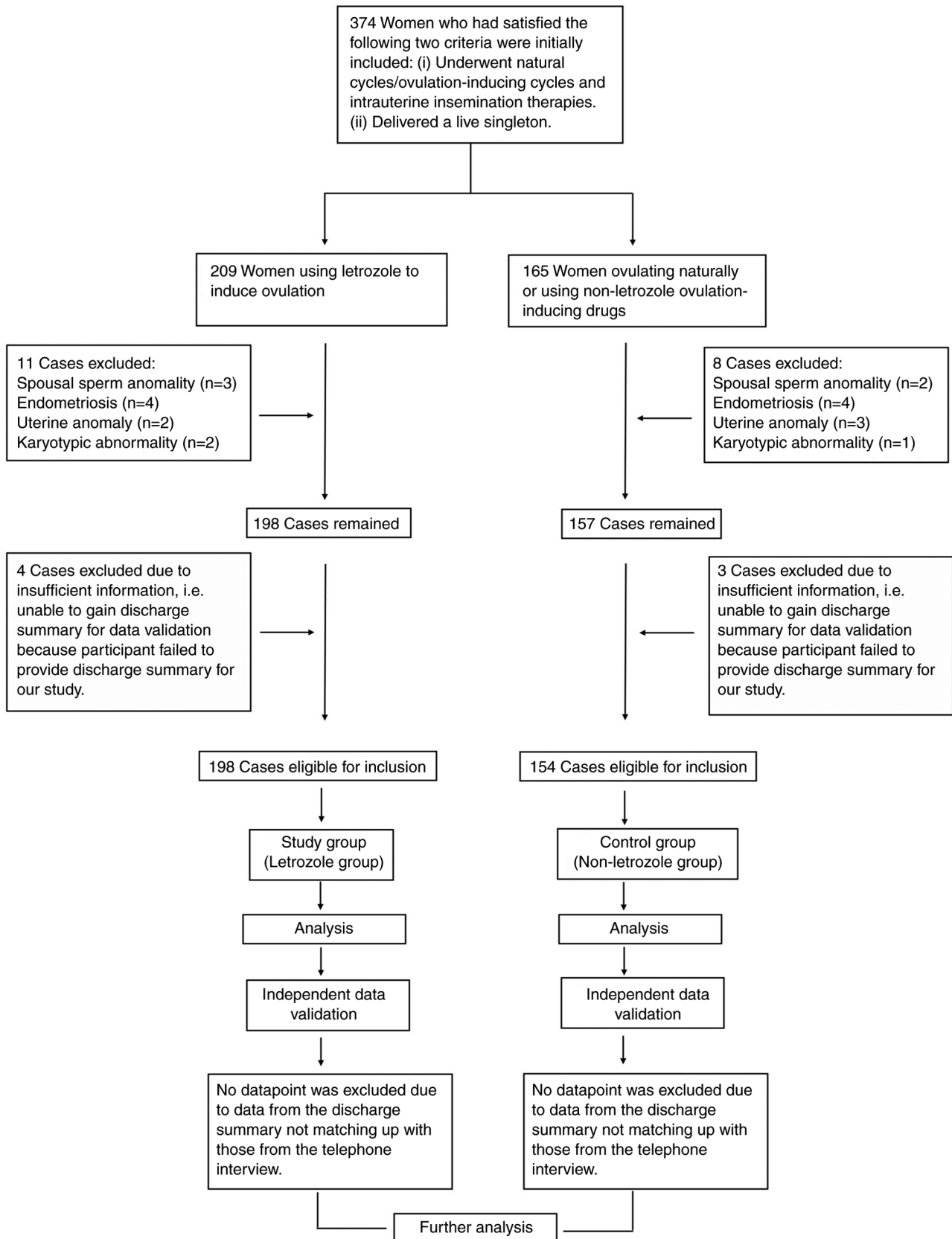


Figure 1. Flowchart describing subject selection and study design.

outcomes via telephone calls. Interview was terminated if one of the following circumstances occurred: i) negative reaction in test for serum  $\beta$ -hCG 2 weeks after the IUI therapy;

ii) miscarriage; and iii) delivery of a live neonate. In the present study, all included participants were adequately followed. The follow-up rate was 100%.

*Independent validation of the data.* In the present study, participants were from four cities (Shantou, Chaozhou, Jieyang and Shanwei) of eastern Guangdong (China). Furthermore, women who came from the three other cities and became pregnant after receiving treatments in The First Affiliated Hospital of Shantou University Medical College, would likely decide to deliver their infants in the nearby hospital of their cities. As a result, some neonates of the present study were born in other hospitals.

Neonates born in The First Affiliated Hospital of Shantou University Medical College and those born in other hospitals (81 in total, 46 in letrozole group and 35 in non-letrozole group) were included in the present study so as to achieve an adequate sample size for analysis. First, for all neonates included in the current study, information about maternal health, pregnancy and neonatal birth outcome were collected via telephone interviews by trained senior nurses. Second, for neonates born in our hospital, data pertaining to pregnancy outcome and neonatal birth outcome were directly extracted from the electronic medical records of the hospital, and were subsequently used for verification against information collected via telephone interview. For neonates born in other hospitals, similar data were acquired from the discharge summary of the puerpera. After verbal informed consent was obtained from the puerpera through phone call, copy of the discharge summary was obtained from the puerpera via electronic mail or regular mail. These collected data were later used for verification.

For each pair of mother and neonate, the data validation was regarded as acceptable if the following three requirements were satisfied: i) Data from medical archives matched up with those from telephone interviews in terms of the basic information of the parents, including name, age, address and contact number. ii) Data from medical archives matched up with those from telephone interviews in terms of neonatal birth outcome, including date of birth, hospital where the delivery occurred, sex, gestational age, mode of delivery, weight, height and Apgar score. Apgar scoring system was used to evaluate the condition of neonates 1 min after birth of the neonates. The numbers were determined by observations of 5 signs (heart rate, respiratory effort, reflex irritability, muscle tone, and color). A rating of 0, 1 or 2 was given to each sign. Apgar score of 10 indicated a good condition for the neonate, while Apgar score <7 indicated a poor condition (16). iii) Data from medical archives matched up with those from telephone interviews in terms of pregnancy outcome, neonatal birth defect (if any) and neonatal complication (if any). If inconformity occurred during the verification, data acquisition was repeated to address the inconformity, data were collected again from another round of telephone interview and the discharge summary was obtained again when necessary, and validation was performed again.

*Neonatal birth outcomes.* Preterm birth was defined as delivery of an infant before 37 weeks of gestation. Low birth weight was defined as the birth of an infant with birth weight <2,500 g. Macrosomia was defined as an infant with birth weight >4,000 g. Fetal growth restriction referred to an infant with birth weight less than the 10th percentile for gestational age. Birth defects were categorized according to the 10th Edition of the Q-code of the International Statistical

Classification of Diseases and Related Health Problems (17). Neonatal complications in the present study included neonatal intensive care unit admission, fetal growth restriction, fetal asphyxia, oligohydramnios, preterm birth, low birth weight, macrosomia, Apgar score <7 and congenital defects.

*Statistical analysis.* Data analyses were conducted with the SPSS program (Version 20.0, IBM Corp.). Proportion data were presented as number or percentage and were compared using Pearson's chi-squared or Fisher's exact test. Continuous data were expressed as the mean  $\pm$  standard deviation or median (minimum-maximum) and compared using unpaired Student's t-test or Mann-Whitney U test, accordingly, depending on the data distribution. Spearman's rank correlation analysis was employed to explore possible correlation between maternal use of letrozole and neonatal birth outcomes. Logistic regression model was constructed to calculate the contributing strength of one specific factor associated with neonatal complications. Missing data were addressed using the listwise deletion method as recommended by SPSS. Statistically significant difference was set at a two-tailed  $P < 0.05$ .

## Results

*Baseline characteristics of women in letrozole and non-letrozole group.* First, the baseline characteristics of women using letrozole to counterparts from women who did not receive letrozole were compared. As shown in Table I, women of the letrozole group were younger than those from the non-letrozole group ( $P < 0.001$  vs. the non-letrozole group). Furthermore, statistically significant difference was observed in the history of previous IUI therapies between the two groups ( $P = 0.049$  vs. the non-letrozole group). However, for other characteristics listed in Table I, data were all comparable between the two groups (all  $P > 0.05$  vs. the non-letrozole group).

*Birth outcomes of singletons in letrozole group and non-letrozole group.* Subsequently, the birth outcomes of singletons in the letrozole and the non-letrozole group were investigated. As indicated in Table II and Fig. 2, a significantly low proportion of caesarean section deliveries were found in the letrozole group (caesarean section deliveries, 43.8% of the letrozole group vs. 56.4% of the non-letrozole group,  $P = 0.019$ ). For other characteristics presented in Table II and Fig. 2, including neonatal sex, full-term or preterm birth, birth weight, birth length, Apgar score and neonatal complications, data were all similar between the two groups ( $P > 0.05$  vs. the non-letrozole group).

*Congenital anomalies of singletons in letrozole group and non-letrozole group.* Moreover, congenital anomalies of singletons in the letrozole group and the non-letrozole group were analyzed. Overall, no significant difference was found between the two groups (Table III and Fig. 3, all  $P > 0.05$  vs. the non-letrozole group). One case of major congenital anomaly (congenital intestinal atresia, required surgical intervention) was reported in the letrozole group, as compared with none in the non-letrozole group (non-significant difference,  $P > 0.999$  vs. the non-letrozole group). In addition, 1 case of minor congenital anomaly (finger

Table I. Baseline characteristics of women in letrozole and non-letrazole group.

Characteristics	Letrozole (n=194)	Non-letrazole (n=154)	P-value
Age (years)	28 (20-41)	29 (21-38)	0.000 <sup>a</sup>
Duration of infertility (years)	3 (1-10)	3 (1-13)	0.381
Etiology of infertility			
Primary	137 (70.6%)	108 (70.1%)	0.921
Secondary	57 (29.4%)	46 (29.9%)	
IUI cycle			
1st cycle	94 (48.7%)	94 (62.2%)	0.049 <sup>a</sup>
2nd cycle	67 (34.7%)	44 (29.1%)	
3rd cycle	24 (12.4%)	9 (5.9%)	
Cycles after 3 attempts	8 (4.1%)	4 (2.6%)	
Body mass index (kg/m <sup>2</sup> )	21.04 (16.8-32.5)	21.35 (13.4-31.6)	0.544
Basal estradiol (pg/ml)	38.0 (14-405)	42.5 (11-125)	0.373
Basal progesterone (ng/ml)	0.32 (0.01-1.39)	0.34 (0.01-1.72)	0.325
Number of mature follicles on the trigger day	1.0 (1-4)	1.0 (1-4)	0.127
Endometrium thickness on the trigger day (mm)	10.0 (5.5-16.0)	10.00 (7.5-15.8)	0.792

<sup>a</sup>Indicates a statistically significant difference. Values are presented as median (minimum-maximum) or number (percentage). IUI, intrauterine insemination.

Table II. Birth outcomes of singletons in letrozole and non-letrazole group.

Characteristics	Letrozole (n=194)	Non-letrazole (n=154)	P-value
Sex			
Male	97 (50%)	76 (49.3%)	0.904
Female	97 (50%)	78 (50.6%)	
Full-term or preterm birth			
Full-term	181 (93.2%)	145 (94.1%)	0.744
Preterm	13 (6.7%)	9 (5.8%)	
Delivery mode			
Spontaneous labor	109 (56.1%)	67 (43.5%)	0.019 <sup>a</sup>
Caesarean section	85 (43.8%)	87 (56.4%)	
Birth weight (g)	3,050 (2,000-4,600)	3,050 (1,900-5,300)	0.599
Birth length (cm)	50 (41-55)	50 (40-54)	0.575
Apgar score			
7~10	193 (99.4%)	152 (98.7%)	0.586
<7	1 (0.5%)	2 (1.2%)	
Singletons with complications <sup>b</sup>			
Yes	26 (13.4%)	28 (18.1%)	0.221
No	168 (86.5%)	126 (81.8%)	

<sup>a</sup>Indicates a statistically significant difference. <sup>b</sup>Complications include neonatal intensive care unit admission, fetal growth restriction, fetal asphyxia, oligohydramnios, preterm birth, low birth weight, macrosomia, Apgar score less than 7 and congenital defects. Values are presented as median (minimum-maximum) or number (percentage).

anomaly) was discovered in the letrozole group, and 3 cases of minor congenital anomalies (1 case of ankylotia, 1 case of undersize right ear and 1 case of strephenopodia) in the non-letrazole group (non-significant difference, P=0.658 vs. the non-letrazole group).

*Correlation between maternal use of letrozole and neonatal birth outcomes.* With Spearman's rank correlation analysis, the possible correlation between maternal use of letrozole and neonatal birth outcomes was explored. As presented in Table IV, after controlling for etiology of infertility, duration



Table III. Congenital anomalies of singletons in letrozole and non-letrozole group.

Congenital anomalies	Letrozole (n=194)	Non-letrozole (n=154)	P-value
Major congenital anomalies	1 (0.51%)	0 (0%)	>0.999
Minor congenital anomalies	1 (0.51%)	3 (2.59%)	0.326
Total congenital anomalies	2 (1.03%)	3 (2.59%)	0.658
Brain and nervous system	0 (0%)	0 (0%)	
Face and neck	0 (0%)	2 (1.29%)	0.195
Circulatory system	0 (0%)	0 (0%)	
Digestive system	1 (0.51%)	0 (0%)	>0.999
Genitourinary system	0 (0%)	0 (0%)	
Anomalies of limbs	1 (0.51%)	1 (0.64%)	>0.999
Chromosomal anomalies	0 (0%)	0 (0%)	

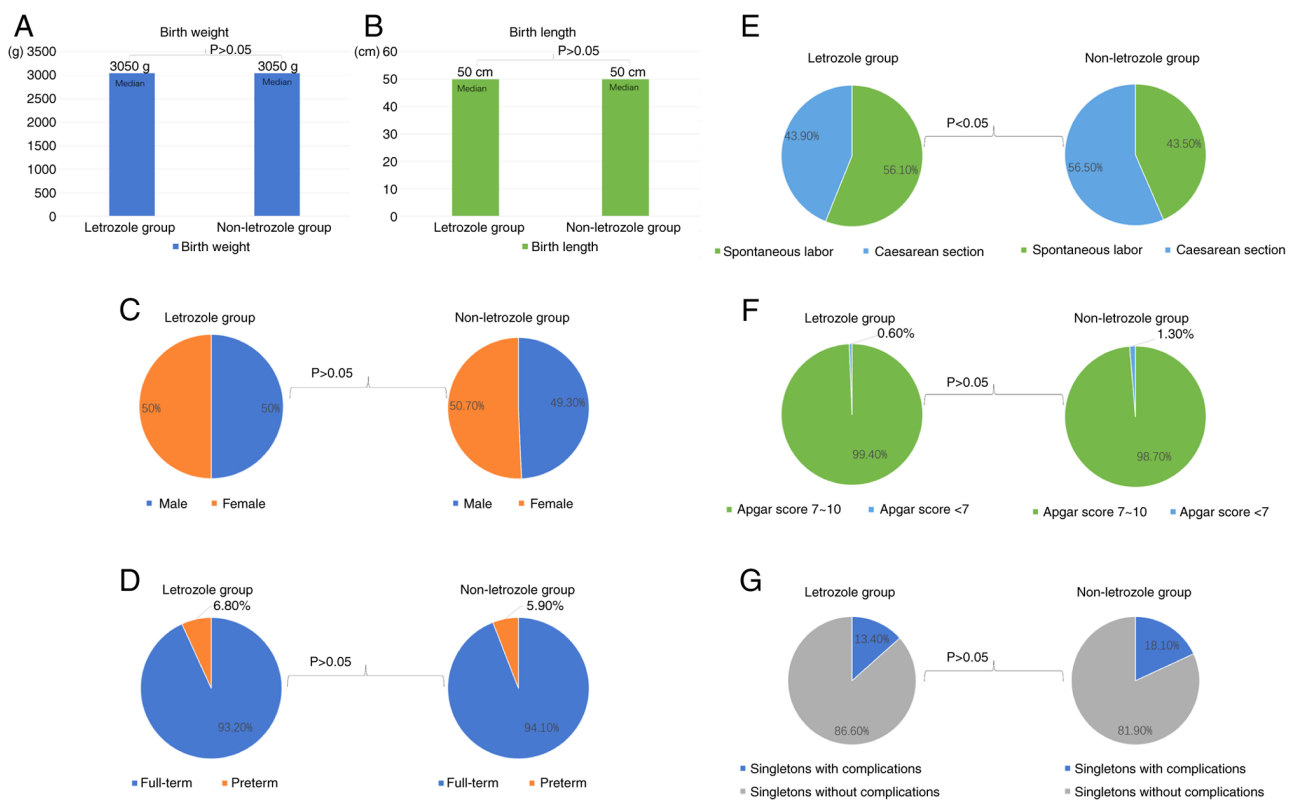


Figure 2. Birth outcomes of singletons in letrozole and non-letrozole group. (A) Birth weight and (B) length; (C) sex; (D) full-term or preterm birth; (E) delivery mode; (F) Apgar score; and (G) singletons with or without complications. Letrozole group, n=194; and non-letrozole group, n=154.

of infertility, age, history of previous IUI and body mass index, no significant correlation was observed between maternal use of letrozole and neonatal birth outcomes (all  $P > 0.05$ ). However, the P-value for the correlation between maternal use of letrozole and neonatal complications was marginal ( $P = 0.051$ ).

*Effect of maternal use of letrozole on neonatal complications of singletons.* Considering the marginal P-value ( $P = 0.051$ ) for the correlation between maternal use of letrozole and neonatal complications, this correlation was further investigated using the logistic regression analysis. As presented in Table V, results from logistic regression analysis confirmed that maternal use of letrozole was not a significant contributor for neonatal

complications, independent of statistical adjustment (crude OR, 1.436; 95% CI, 0.803-2.569;  $P = 0.223$  vs. adjusted OR, 1.406; 95% CI, 0.748-2.643;  $P = 0.290$ ).

## Discussion

Currently, the investigation of the safety of letrozole administration for ovulation induction is limited in the Chinese cohort. The present study is the first report of such kind. Overall, it was found that birth outcomes of neonates born to mothers using letrozole were not inferior when compared with neonates born to mothers using non-letrozole ovulation-inducing strategies. This finding indicated that maternal use of letrozole is not associated

Table IV. Correlation analysis between maternal use of letrozole and neonatal birth outcomes.

		n=348						
Investigated factors		Delivery mode	Full-term or preterm birth	Neonatal sex	Birth length	Birth weight	Apgar score	Neonatal complications <sup>a</sup>
Letrozole use to induce ovulation <sup>b</sup>	Correlation coefficient	0.059	0.066	0.033	0.071	0.012	0.068	0.126
	P-value	0.361	0.306	0.604	0.270	0.852	0.290	0.051

<sup>a</sup>Complications include neonatal intensive care unit admission, fetal growth restriction, fetal asphyxia, oligohydramnios, preterm birth, low birth weight, macrosomia, Apgar score <7 and congenital defects. <sup>b</sup>Controlled for etiology of infertility, duration of infertility, age, history of previous intrauterine insemination and body mass index.

Table V. Effect of maternal use of letrozole on neonatal complications of singletons.

Factors investigated	Neonatal complications of singletons <sup>a</sup> (n=348)			
	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Letrozole use to induce ovulation <sup>b</sup>	1.436 (0.803-2.569)	0.223	1.406 (0.748-2.643)	0.290

<sup>a</sup>Complications include neonatal intensive care unit admission, fetal growth restriction, fetal asphyxia, oligohydramnios, preterm birth, low birth weight, macrosomia, Apgar score <7 and congenital defects. <sup>b</sup>Adjusted for etiology of infertility, duration of infertility, age, history of previous intrauterine insemination and body mass index. OR, odds ratio; CI, confidence interval.

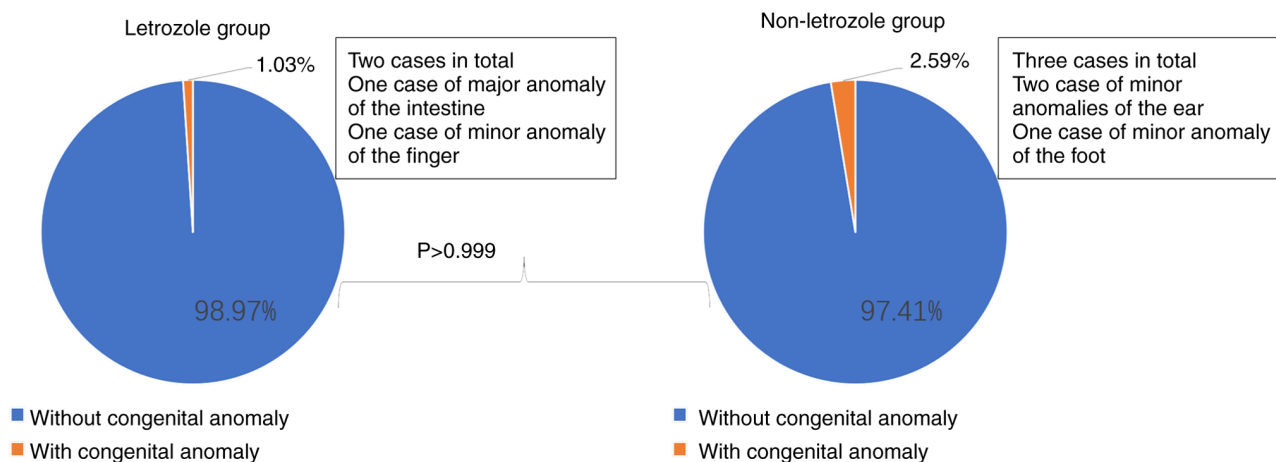


Figure 3. Congenital anomalies of singletons in letrozole and non-letrozole group.

with poorer birth outcomes, which is consistent with numerous previous studies (1,10-13). Noteworthy, the percentage of caesarean section deliveries in the letrozole group was significantly lower than that of the non-letrozole group (Table II). This phenomenon may be partly attributed to the younger age of women in the letrozole group (Table I), since previous studies have demonstrated that advanced age of puerpera is associated with elevated possibilities of a caesarean section delivery (18).

As one of the important aims of the present study, the incidence of congenital anomalies between the letrozole and the non-letrozole group was evaluated. It was discovered that incidence of major and minor congenital anomalies between the two groups were comparable (Table III). This finding

supported the fact that, as an ovulation-inducing drug, letrozole is equally safe when compared with other counterparts. Maternal use of letrozole does not appear to be associated with increased risk of congenital anomaly. This finding is in agreement with numerous previous studies (8).

In the present study, a case of major congenital anomaly (congenital intestinal atresia) and a case of minor congenital anomaly (finger anomaly) were observed in the letrozole group. By contrast, a total of 3 cases of minor congenital anomalies (a case of ankylotia, a case of undersized right ear and a case of strephenopodia) were observed in the non-letrozole group. The overall incidence of congenital anomaly in the letrozole and the non-letrozole group were 1.03 and 2.59% respectively,

which are lower than those observed in the general population (19). This discrepancy may be due to the fact that only live singletons were included and analyzed in the present study. Congenital anomalies of twins or triplets were excluded in the present study to avoid any biases that originated from multiple births. This exclusion inevitably reduced the incidence of congenital anomaly observed herein.

Despite numerous previous studies having investigated the impact of maternal use of letrozole on congenital anomaly, the relationship between maternal use of letrozole and neonatal complications still remains largely unknown (1,10-13). Therefore, as another important purpose of the present study, the relation between maternal use of letrozole and neonatal complications was analyzed. With Spearman's rank correlation analysis, a marginal P-value ( $P=0.051$ ) for the correlation between maternal use of letrozole and neonatal complications was found (Table IV). To further clarify this ambiguity, the authors went on validating this correlation using the logistic regression model. Results from both unadjusted and adjusted regression analyses revealed that maternal use of letrozole is not a significant predictor for neonatal complications (Table V). These findings appear to suggest that maternal use of letrozole for ovulation does not correlate with increased risk of neonatal complications. These findings provided us with additional evidences to support the safety of letrozole as an ovulation-inducing drug.

At present, the major concern about application of letrozole as an ovulation-inducing drug centers on its debatable teratogenic effects (6). However, for a teratogenic effect to occur, teratogen must be present at the time of embryogenesis or organogenesis (4,5). The median half-life of letrozole is ~45 h (30-60 h) (4). Generally, letrozole is administrated in the early follicular phase to induce ovulation, and it should be completely eliminated from the body by the time of embryogenesis or organogenesis. It is unlikely that letrozole may directly affect fetal embryogenesis or organogenesis (20). The findings of the present study are in agreement with this theory and indicate that letrozole could be a safe drug for ovulation induction.

The present study has its own strengths and limitations. As congenital anomalies were more prevalent among multiple births (10). Only neonatal data of live singletons were included for analysis in the study, while those from multiple gestations were excluded so as to avoid any possible biases originated from multiple gestations. In addition, unlike multicenter studies, in the present study all the pregnancy-related attempts were conducted and completed in the same hospital by the same medical team to ensure the consistency of the data and minimize potential biases as much as possible.

However, there are limitations to the present study. One of the limitations is the retrospective design of the study. It is likely that recall bias or misinterpretation bias may occur during interview, as information collected through interview may not always be accurate. To address this problem, data were obtained again from medical archives and subsequently used for verification against those collected from the interview. This validation procedure was carried out to reduce the influence of recall bias or misinterpretation bias. However, owing to the retrospective design of the present study, additional prospective studies are needed to further confirm the current finding. The second limitation is the relatively small sample

size; further researches with sufficient sample size are necessary to confirm the findings of the present study.

In conclusion, the results appeared to suggest that maternal use of letrozole for ovulation induction does not associate with poorer birth outcomes or increased risk of congenital anomalies and neonatal complications. The present study provided additional evidence to support the safety of letrozole as an ovulation-inducing drug, and helped to restore letrozole as a low-risk medicine for ovulation induction by reducing the concern about its teratogenic effects.

## Acknowledgements

Not applicable.

## Funding

The present study was supported by Guangdong Science and Technology Department (grant no. 2016A020218015).

## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

BW and ZL designed the study. BW, RX, HL and SL collected and analyzed the data. BW, HL and SL drafted the initial manuscript. ZL reviewed and edited the article. ZL and SL served as guarantors, accepted full responsibility for the work and controlled the decision to publish. All authors read and approved the final manuscript. BW and ZL confirmed the authenticity of all the raw data.

## Ethics approval and consent to participate

The present study was approved (approval no. 2015) by the Institutional Ethics Committee of The First Affiliated Hospital of Shantou University Medical College (Shantou, China). Written informed consent was provided by all participants. The present study was carried out according to the principles of The Declaration of Helsinki.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Takeshima K, Ezoe K, Kawasaki N, Hayashi H, Kuroda T and Kato K: Perinatal outcomes and congenital anomalies associated with letrozole and natural cycles in single fresh cleaved embryo transfers: A single-center, 10-year cohort study. *F S Rep* 3: 138-144, 2022.
2. Gonen Y and Casper RF: Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. *Hum Reprod* 5: 670-674, 1990.



3. Weiss NS, van Vliet MN, Limpens J, Hompes PGA, Lambalk CB, Mochtar MH, van der Veen F, Mol BWJ and van Wely M: Endometrial thickness in women undergoing IUI with ovarian stimulation. How thick is too thin? A systematic review and meta-analysis. *Hum Reprod* 32: 1009-1018, 2017.
4. Tulandi T and DeCherney AH: Limiting access to letrozole-is it justified? *Fertil Steril* 88: 779-780, 2007.
5. Kar S: Current evidence supporting 'letrozole' for ovulation induction. *J Hum Reprod Sci* 6: 93-98, 2013.
6. Biljan MM, Hemmings R and Brassard N: The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. *Fertil Steril* 84 (Suppl 1.1): S95, 2005.
7. Casper RF and Mitwally MF: A historical perspective of aromatase inhibitors for ovulation induction. *Fertil Steril* 98: 1352-1355, 2012.
8. Pundir J, Achilli C, Bhide P, Sabatini L, Legro RS, Rombauts L, Teede H, Coomarasamy A, Zamora J and Thangaratinam S: Risk of foetal harm with letrozole use in fertility treatment: A systematic review and meta-analysis. *Hum Reprod Update* 27: 474-485, 2021.
9. Yang AM, Cui N, Sun YF and Hao GM: Letrozole for female infertility. *Front Endocrinol (Lausanne)* 12: 676133, 2021.
10. Sharma S, Ghosh S, Singh S, Chakravarty A, Ganesh A, Rajani S and Chakravarty BN: Congenital malformations among babies born following letrozole or clomiphene for infertility treatment. *PLoS One* 9: e108219, 2014.
11. Akbari Sene A, Ghorbani S and Ashrafi M: Comparison of the pregnancy outcomes and the incidence of fetal congenital abnormalities in infertile women treated with letrozole and clomiphene citrate. *J Obstet Gynaecol Res* 44: 1036-1041, 2018.
12. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, Librach C, Greenblatt E and Casper RF: Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil Steril* 85: 1761-1765, 2006.
13. Yun J, Choi YS, Lee I, Won YB, Lee JH, Seo SK, Cho S, Lee BS and Yun BH: Comparison of congenital malformations among babies born after administration of letrozole or clomiphene citrate for infertility treatment in a Korean cohort. *Reprod Toxicol* 82: 88-93, 2018.
14. Wang B and Li Z: Comparison of dual-trigger and human chorionic gonadotropin-only trigger among polycystic ovary syndrome couples who underwent controlled ovarian stimulation and intrauterine insemination: A retrospective cohort study. *Medicine (Baltimore)* 102: e32867, 2023.
15. Wang B and Li Z: Hypersecretion of basal luteinizing hormone and an increased risk of pregnancy loss among women with polycystic ovary syndrome undergoing controlled ovarian stimulation and intrauterine insemination. *Heliyon* 9: e16233, 2023.
16. Papile LA: The Apgar score in the 21st century. *New Engl J Med* 344: 519-520, 2001.
17. World Health Organization (WHO): International statistical classification of diseases and related health problems. 10th Revision, fifth edition. WHO, Geneva, 2016. <https://apps.who.int/iris/handle/10665/246208>. Accessed August 27, 2023.
18. Marchi J, Berg M, Dencker A, Olander EK and Begley C: Risks associated with obesity in pregnancy, for the mother and baby: A systematic review of reviews. *Obes Rev* 16: 621-638, 2015.
19. Jacqz-Aigrain E and Koren G: Effects of drugs on the fetus. *Semin Fetal Neonatal Med* 10: 139-147, 2005.
20. Dean JCS: Catalog of teratogenic agents. *J Med Genet* 39: 454, 2002. .



Copyright © 2024 Wang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.