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Impact of inoculation of inactivated COVID-19 vaccine on the outcomes of frozen-thawed embryo transfer

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Article

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Abstract

To assess the impact of inoculation of inactivated corona virus disease 2019 (COVID-19) vaccine on the outcome of frozen-thawed embryo transfer (FET). From January 2022 to November 2023, patients aged 20 ~ 45 years old undergoing FET at the Reproductive Medicine Center of a tertiary teaching hospital were prospectively enrolled. The patients were divided into vaccinated group (n = 458) and unvaccinated group (n = 530) based on the inoculation of inactivated COVID-19 vaccine before FET. Vaccinated group was further divided into three subgroups based on the dose (single dose, n = 55; double dose, n = 292; triple dose, n = 111) or interval from the first inoculation to FET (< 3 months, n = 51; 3 ~ 6 months, n = 101; > 6 months, n = 306). The primary outcome was live birth rate (LBR). The LBR (43.87% *vs.* 40.57%) was not significantly different between the vaccinated and unvaccinated groups, and so were embryo implantation rate (IR), clinical pregnancy rate (CPR), the gestational age at delivery and birth weight ($P > 0.05$). IR was significantly decreased with the shorter interval of vaccination (28.57% *vs.* 32.02% *vs.*45.24%, $P = 0.007$), while LBR and CPR were not significantly different ($P > 0.05$). For the dose subgroups of vaccination, IR, LBR and CPR were not significantly different ($P > 0.05$). Inoculation of inactivated COVID-19 vaccine did not affect the outcome of FET.

Clinical Trial Registration Number: ChiCTR2200055597 (Chinese Clinical Trial Registry), January 14, 2022. (https://www.chictr.org.cn/bin/project/edit?pid=148312)

Introduction

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since the end of 2019 had a huge and far-reaching impact on the global public health and economic development. To date, a total of 775 million cases of corona virus disease 2019 (COVID-19) have been confirmed, which resulted in at least 7.05 million deaths worldwide [1]. Infection of SARS-CoV-2 may cause multisystem diseases, and the virus or RNA of virus has been detected in a variety of body fluids including saliva, urine, feces, semen and vaginal fluid [2–4]. Some enzymes expressed in the reproductive system, such as angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine isoform 2 (TMPRSS2), may participate in the pathological processes of the infection with SARS-CoV-2 [5]. To enter the cell, SARS-CoV-2 will use its spike S protein to bind with ACE2, which will lead to fusion with the cell membrane and endocytosis. TMPRSS2, a transmembrane protease, cleaves the S protein. And cleavage is necessary for the virus to bind with ACE2 and spread through the infected host [6]. Cathepsin B (CTSB), cathepsin L (CTSL) and FURIN, which are expressed in the endometrium, can also cleave the S protein and enhance the SARS-CoV-2 infectivity in the endometrium [6]. Therefore, the reproductive system is involved in the infection of SARS-CoV-2. Severe illness and death, and Cesarean delivery have been more among pregnant women infected with SARS-CoV-2 [7]. Therefore, females should avoid infection with SARS-CoV-2 before conception, and inoculation is an important and effective method.

By November 2023, the coverage rate of inoculation of COVID-19 vaccine (at least two doses) had reached 56% globally and 87% in China [8]. The type of COVID-19 vaccines has, included inactivated,

mRNA, DNA, non- replicating viral vector, live attenuated, subunit, and trained immunity-based vaccine [9]. The efficacy and safety of COVID-19 vaccine have been reported in pregnant women and fetus from *in vitro* fertilization and embryo transfer (IVF-ET) [10, 11]. As the main type of vaccine in China, inactivated COVID-19 vaccine was reported to be safe and effective for the general population, including older generation [12], children and adolescents [13], pregnant women, infertility patients undergoing IVF-ET and intrauterine insemination [14]. Antibodies to COVID-19 vaccine have been found in cord blood and breast milk following inoculation, which suggested that maternal inoculation could protect the fetus [15].

The International Committee for Monitoring Assisted Reproductive Technology (ICMART) has reported that the cycles of frozen-thawed embryo transfer (FET) have been more commonly used than the fresh embryo transfer globally in 2020 [16]. Whereas, reports about inoculation of COVID-19 vaccine in patients undergoing FET have been rare. Two retrospective studies have reported that the endometrial receptivity, embryo implantation rate (IR) and live birth rate (LBR) were not affected by the inactivated COVID-19 vaccine, though the vaccination dose or information of triple dose were not reported [17, 18]. In China, the full course of inactivated COVID-19 vaccination was consisted of three doses: the first dose, the second dose given one month later after the first dose, and the third dose (booster dose) given six months later after the second dose. Therefore, the triple dose should be considered for analysis. The duration of inoculation with COVID-19 vaccine was mainly from April 2021 to January 2023 in China. Thus, almost all pregnant women had been inoculated with the COVID-19 vaccine before FET were delivered. Therefore, in this study, we compared the LBR and other outcomes in patients inoculated with COVID-19 vaccine before FET, including between different dose and intervals.

Methods

Ethical statements

This prospective cohort study has been registered with the Chinese Clinical Trial Registry Centre (Registration No. ChiCTR2200055597) and approved by the Medical Ethics Committee of Sichuan Provincial Women's and Children's Hospital. Signed informed consent forms were obtained from all participants. All procedures have complied with the ethical standards of relevant national and institutional committees on human experimentation and the Helsinki Declaration 1975 (2013 revision).

Study populations

From January 2022 to November 2023, patients aged 20 ~ 45 undergoing FET at the Reproductive Medicine Center of Sichuan Provincial Women's and Children's Hospital were prospectively enrolled. The exclusion criteria had included infection with SARS-CoV-2 before FET, abnormal female reproductive conduct (congenital uterine malformation, submucosal or intramural uterine fibroids, adenomyosis), repeated implantation failure, preimplantation genetic testing, inaccurate information of vaccination. Patients were divided into vaccinated group and unvaccinated group based on inoculation of inactivated COVID-19 vaccine before FET. The vaccinated group was divided into three subgroups based on the dose

(single, double and triple dose) or interval from the first inoculation to FET (< 3 months, 3 ~ 6 months, > 6 months).

Personal information was recorded into the electronic database of the Reproductive Center. In Sichuan province, the date of vaccination, the type and manufacturer of vaccine could be queried through Tianfu Tong (an application program of Sichuan health database) installed on the mobile phone. Nurses will record the information of vaccination of the patient on the day of FET. All patients were routinely detected for nucleic acid to exclude SARS-CoV-2 infection before FET. The antral follicle count (AFC), body mass index (BMI), and serum level of anti-Müllerian hormone (AMH) were measured as described previously [19].

After ovarian stimulation and oocyte retrieval, IVF or intracytoplasmic sperm injection (ICSI) were conducted in a culture environment containing 6.5% CO_2 and 5% O_2. Then fertilized oocytes were cultured in sequential G1-plus/G2-plus medium (Vitrolife, Sweden). Day 3 cleavage embryos and blastocysts were cryopreserved using Kitazato vitrification solution at room temperature [20].

Endometrial preparation protocols and frozen-thawed embryo transfer

At least one months after embryo cryopreserved, FET was conducted by one of the three endometrial preparation protocols. For hormone replacement therapy (HRT) protocol, estradiol valerate (Progynova, Bayer, Germany) was orally took 6 mg daily since day 2 ~ 3 of menstruation for at least 14 days, then the progesterone oil (Xianju, Zhejiang, China) was intramuscular injected 60 mg daily when the endometrial thickness > 7 mm. For natural cycle protocol, follicle development and endometrial growth was monitored with ultrasonography without medicine. For induced ovulation cycle, letrozole (Hengrui, Jiangsu, China) was orally took 5 mg daily for 6 days. Progesterone oil (Xianju, Zhejiang, China) was intramuscular injected 40 mg since ovulation daily both in natural cycle and induced ovulation cycle. For the three protocols, cleavage embryo or blastocyst was thawed and transferred on fourth or sixth day of progesterone oil injection, respectively. One or two embryo was Then luteal phase support was added with dydrogesterone (Abbott, The Netherlands) 30 mg daily and vaginal progesterone gel 90 mg/day (Crinone, Merck, Germany) after FET in all endometrial preparation protocols.

Outcomes of FET

Serum human chorionic gonadotropin (hCG) was measured 10 or 12 days after cleavage or blastocyst FET, respectively. Pregnancy was considered when hCG > 5 IU/mL. Clinical pregnancy was defined as demonstration of a gestational sac with an embryo showing cardiac activity. Early miscarriage and late miscarriage were defined as loss of pregnancy before 12 or 12 ~ 28 weeks of gestation, respectively. Ectopic pregnancy was defined as embryo implantation in any other site out of the uterine cavity. Ongoing pregnancy was defined as detection of fetal heartbeat at 12 weeks of gestation. Live birth was defined as delivery of live newborn at least over 28 weeks of gestation.

The primary outcome was LBR. The secondary outcomes were IR (ratio of the number of gestational sac to the number of embryos transferred), clinical pregnancy rate (CPR), early miscarriage rate and ongoing pregnancy rate.

Statistical analysis

SPSS 26.0 software was used for statistical analysis. Kolmogorov-Smirnov test was used to assess the normality of quantitative data. Normally distributed quantitative data were expressed as mean ± standard deviation (M ± SD) and analyzed by one-way ANOVA. Non-normally distributed quantitative data were expressed as median and interquartile range, and analyzed by the Non-parametric test. Multivariate binary logistic regression analysis was performed to evaluate the independent effect of Covid-19 vaccination status on reproductive outcomes adjusting for potential confounding variables. Data was presented as adjusted odds ratios (aOR) and 95% CI (Confidence interval), as well as significance (P value). Qualitative data were expressed as frequency, and compared by chi-square or Fisher's exact tests. Bonferroni's method was conducted for multiple testing. Two-tailed $P < 0.05$ was considered as statistically significant.

Results

Baseline characteristics of the study population

The flow diagram of participant selection was shown in Fig 1. After exclusion of 308 patients, 458 and 530 patients were included in the vaccinated and unvaccinated groups, respectively. The patients in the vaccinated group were all inoculated the inactivated COVID-19 vaccine was produced by Beijing Sinovac Biotechnology Co., LTD or Chengdu Institute of Biological Products.

The age, duration of infertility, BMI, AFC, serum level of AMH, endometrial thickness, number of embryos transferred; proportions of infertility type, infertility etiology, type of fertilization, protocol of endometrial preparation, blastocyst were not significantly different between vaccinated and unvaccinated groups ($P > 0.05$) (Table 1).

Outcomes of FET between the vaccinated and unvaccinated groups

The rates of implantation (38.16% *vs.* 36.64%), clinical pregnancy (52.18% *vs.* 50.00%), early miscarriage (10.04% *vs.* 12.08%), ectopic pregnancy (1.67% vs. 2.26%), ongoing pregnancy (46.07% *vs.* 42.83%), multiple pregnancy (14.64% *vs.* 18.49%), late miscarriage (4.18% *vs.* 4.15%) and live birth (43.87% *vs.* 40.57%) were not significantly different between the vaccinated and unvaccinated groups ($P > 0.05$) (Table 2). Adjusting for potential confounding variables, logistic regression analysis results showed no statistically differences of adjust odds ratio for CPR, LBR and ongoing pregnancy rate between vaccinated and unvaccinated women ($P > 0.05$) (Table 3).

All multiple pregnancies were twin pregnancies. There was one stillbirth from the unvaccinated group. Of 494 (267 boys and 227 girls) live-born infants, one infant from the unvaccinated group had heart

malformation. The mean gestational age at delivery was 38 weeks (29 ~ 41 weeks) and the mean birth weight was 3035 g (1150 ~ 4440 g).

Outcomes of FET in the Subgroup analysis of vaccinated women

For the vaccinated group, the dose subgroups were single dose (n = 55), double dose (n = 292) and triple dose (n = 111), and interval subgroups were < 3 months (n = 51), 3 ~ 6 months (n = 101) and > 6 months (n = 306), respectively (Table 4).

CPR, LBR, and rates of early miscarriage, ectopic pregnancy, ongoing pregnancy and late miscarriage were not significantly different by both dose and interval subgroup analysis ($P > 0.05$). IR was significantly lower with shorter interval of inoculation (28.57% *vs.* 32.02% *vs.* 42.54%, $P = 0.007$), whereas no significant difference was found by dose subgroup analysis ($P > 0.05$).

Discussion

In this study, we have found that LBR, CPR, gestational age at delivery and birth weight were similar between the vaccinated and unvaccinated groups, as well as between the dose and interval of the vaccinated subgroups, except that the IR was increased along with longer interval of inoculation before FET.

In keeping with our report, other studies have also found that CPR, ongoing pregnancy rate and LBR of FET were similar between vaccinated and non-vaccinated groups [17, 18]. Therefore, we propose that the inoculation of inactivated COVID-19 vaccine before FET is safe and will not affect the endometrial receptivity. By dose subgroup analysis (single, double and triple dose), we also found that IR, CPR, ongoing pregnancy, early miscarriage, ectopic pregnancy, LBR, gestational age at delivery and birth weight were not significantly different. Cao *et al*. [18] reported that LBR was not significantly different between those receiving single and double dose of inactivated COVID-19 vaccine before FET (53.5% *vs.* 43.0%), though they did not include those receiving triple doses. For mRNA COVID-19 vaccine, studies also reported that the dose of inoculation did not affect the endometrial receptivity, IR, CPR and LBR, and the complications of pregnancy and newborn (such as hypertensive disorders, small for gestational age, and respiratory complications) [21, 22].

We found that IR was decreased with the shorter interval of inoculation of inactivated COVID-19 vaccine, which suggested that early stage of inoculation may affect endometrial receptivity. The phenomenon may be associated with immune status of endometrium after inoculation. Vaccination is an immune process with inflammatory response including COVID-19 vaccination [23, 24]. Immune status of the endometrium was closely associated with endometrial receptivity [25]. Therefore, we propose that the endometrial receptivity is decreased closing inoculation with COVID-19 vaccine with cautious because small sample size (51 patients) in the sub-group (< 3 months). While Brandao *et al.* [22] reported that the interval of inoculation with COVID-19 mRNA vaccine did not affect the endometrial receptivity and

embryo implantation in FET with single euploid blastocyst. The different immune response followed by different type of vaccine might be the reason [26].

LBR is more important than IR with regard to the outcome of FET. Both Cao's [18] and our study have found that LBR and birth weight were not significantly different by interval subgroup analysis. Hence, the impact of inactivated COVID-19 vaccine on the endometrial receptivity may not be sufficient to affect the LBR and infants. So far, no consensus has been reached over the interval between vaccinations and ART. Guidelines in China has recommended one month [27], while European Society of Human Reproduction and Embryology has suggested two months [28]. Shi *et al.* [29] suggested at least 61 days. Cao *et al.* [18] suggested that to postpone FET and conception plan for over 3 months due to Covid-19 vaccination was not necessary. Even IR was decreased as shorter interval between inoculation and FET, LBR was not affected in our study. Therefore, we suggested that patients need not to postpone FET after inoculation of inactivated COVID-19 vaccine more than 3 months.

The limitations of this study include the small sample size in the dose and interval subgroups, especially in single dose and < 3 months subgroups. All patients have come from a single center. Cleavage embryos and blastocysts were mixed together for analysis due to small sample size. Therefore, the outcomes should be interpreted with caution. Further studies with larger sample size and involve multiple centers are required to validate the conclusion.

Conclusion

Inoculation of inactivated COVID-19 vaccine did not affect the outcome of FET. Patients need not to postpone FET after inoculation of inactivated COVID-19 vaccine more than 3 months from our suggestion.

Declarations

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Authors' contributions

Yu Qiu wrote the manuscript. Yan Gong designed the study and revised the article. Shi-qi Chen and Jia-jing Wei contributed to data analyzing. Mei Leng and Xi Deng contributed to patient enrollment. Mei-yu Liang and Rong-ning Ma contributed to patient follow-up. Fu-rui Chen and Jing Hei collected the data. Jesse Li-Ling revised the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Sichuan Provincial Women's and Children's Hospital. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

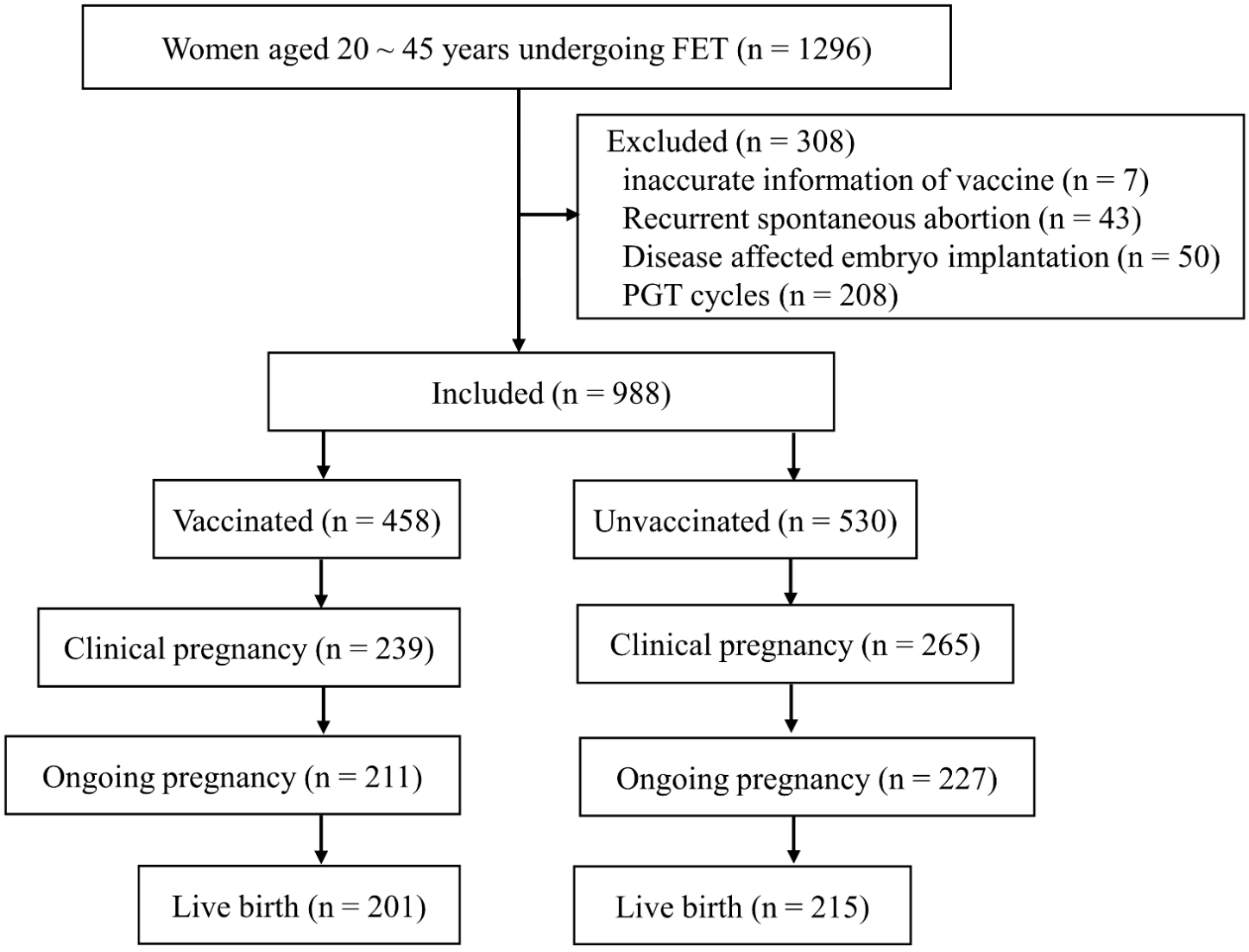


Figure 1

Flow diagram of participant selection.

Description: Progression from recruitment to completion.

Supplementary Files

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