Running title: Frozen embryo transfer after cesarean section

Title: Hormone replacement therapy may increase the risk of early miscarriage during frozen embryo transfer cycles in patients with a history of cesarean section: a retrospective cohort study

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Tweetable abstract

This retrospective study found that a history of caesarean section in patients undergoing frozen embryo transfer using hormone replacement therapy for endometrial preparation was associated with higher early miscarriage rates.

Abstract

Objective: To investigate the efficacies of three cycle regimens in women receiving FET with a history of CS: natural cycle (NC) treatment, hormone replacement therapy (HRT) and treatment with gonadotropin-releasing hormone agonist (GnRH-a) + HRT).

Design: Retrospective cohort study.

Setting: University-affiliated center.

Population: Patients (N = 6,159) with a history of CS who fulfilled the inclusion criteria were enrolled in the study from January 2014 to December 2019.

Methods: Reproductive outcomes of patients in the NC (n = 4,306) versus HRT (n = 1,007) versus GnRH-a + HRT groups (n = 846) were compared.

Main Outcome Measure: The main outcome measure was the live birth rate per embryo transfer (ET).

Results: The unadjusted odds of the miscarriage rate of singleton pregnancies were also significantly higher in the HRT-group compared with the NC-group (25.5% versus 20.4%, respectively). After adjusting for possible confounding factors, the early miscarriage rate and the miscarriage rate of singleton pregnancies remained significantly higher in the HRT-group than the NC-group. The clinical pregnancy rates in the NC-, HRT- and GnRH-a + HRT-groups of women with a history of CS was 48.8%, 48% and 47.1%, respectively, and the live birth rates were 37%, 34.1% and 35.7%, respectively.

Conclusion(s): In women undergoing FET with a history of CS, HRT for endometrial preparation was associated with a higher early miscarriage rate, albeit after statistical adjustment for confounding factors.

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Key Words: Caesarean section, endometrial preparation, frozen embryo transfer, miscarriage

Introduction

In 160 countries surveyed, 29.7 million (21·1%) live births were delivered via cesarean section (CS) in 2015, almost doubling the prevalence of CSs performed in 2000 (12.1%)[1]. More than 30% of births in the USA and Australia[2, 3], and 40% to 50% in China and Brazil[4, 5] were delivered by CS. These trends are driven by older age, pregnancy complications, requests for CS, commercial reasons, litigation and assisted reproductive technology (ART),[6-8] which is associated with greater odds of CS delivery compared to the routine prenatal care of fertile women[6, 9].

CS is a risk factor for lower rates of fertility/infertility and early miscarriage, including ectopic pregnancy and spontaneous abortion[10]. Lower pregnancy, implantation rates and live births after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI)[11-13]may be due to intracavitary fluid (ICF) from hormonal stimulation for controlled ovarian stimulation (COS) in patients with an isthmocele after a CS[14].

In 2015, the Chinese government introduced the second-child policy. Hence, many women with a history of a CS delivery conceived through ART are in need of frozen embryo transfer (FET) for their second progeny. It is necessary for women with secondary infertility and a CS history to undergo frozen embryo transfer because of the adverse effects of COS in patients with previous CS. Various protocols for endometrium preparation have been used to provide an optimal uterine environment for the transfer of thawed embryos, but the evidence supporting the superiority of one protocol over another is insufficient[15, 16]. Therefore, the purpose of the present study was to compare the pregnancy outcomes following different FET protocols among women who have undergone CS.

Materials and Methods

Study Design and Participants

A retrospective study was conducted at the CITIC-Xiangya Hospital of Reproduction and Genetics. The Institutional Ethics Committee of the Central South University approved the study's protocol（2021-KT50）.

Infertile women with a history of CS undergoing FET cycles were enrolled from January 2014 to December 2019. The exclusion criteria were: > 40 years of age at oocyte retrieval, history of multiple CSs, recurrent spontaneous abortion, recurrent implantation failure, preimplantation genetic testing, previous uterine myomectomy or operative hysteroscopy for intrauterine adhesions, untreated hydrosalpinx, adenomyosis, autoimmune or endocrine disease or missing records in the electronic database. Based on their endometrial protocols, the women included in the study were divided into three groups: (i) natural cycle (NC), (ii) hormone replacement therapy (HRT) and (iii) gonadotropin-releasing hormone agonist (GnRH-a) + HRT-groups.

Endometrial Preparation before Embryo Transfer

The decision to proceed with the NC-, HRT- or GnRH-a-group was determined by physician guidance and patient preference. All women were screened for endometrial thickness using transvaginal sonography, and blood samples were taken to measure luteinizing hormone (LH), estradiol and progesterone levels before performing the FET.

Participants in the NC-group did not take any medication throughout the follicular phase, which has been similarly described in work published by our group[17].

Participants in the HRT-group began taking oral estradiol valerate (Progynova, Delpharm Lille SAS, France) on the third day of a natural or progesterone-induced menstrual cycle. The drug was administered either as a fixed dose (6 mg daily) or an incremental dose (2 to 6 mg daily). A vaginal ultrasound examination was conducted 10–15 days later to measure endometrial thickness and ensure that no dominant follicle emerged. When endometrial thickness reached 8 mm, dydrogesterone (10 mg per 12 h; Duphaston, Abbott Biologicals BV, The Netherlands) and the progesterone medication, Utrogestan (200 mg, three times a day; France) were administered orally and vaginally, respectively, to provide luteal phase support until 10 weeks of gestation if a pregnancy had occurred. Embryo transfer (ET) was performed three days after dydrogesterone and progesterone were administered for the day-3 embryos or five days later for blastocysts. If the endometrial thickness was < 8 mm, the estrogen dosage was increased to 8 mg/d for another week. If the endometrial thickness remained inadequate, the cycle was cancelled.

Participants in the GnRH-a + HRT-group received a depot injection of long-acting GnRH-a Triptorelin (1.875 mg, Ferring GmbH, Kiel, Germany). Twenty-one days after receiving the GnRH-a injection, the women underwent ultrasound examinations and blood tests to measure their levels of serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol to confirm complete pituitary downregulation before beginning exogenous hormone supplementation, which commenced on the third day without bleeding with a referral to HRT-FET.

Embryo Vitrification, Thawing and Transfer

Embryos were vitrified using the Kitazato Embryo Vitrification Kit (Kitazato Biopharma

Co, Ltd) using high-security vitrification straws (Cryo Bio System). The embryos were transferred to a commercially available warming solution for thawing (Kitazato Biopharma), following the manufacturer's instructions.

Cleavage-stage embryos (day 3) were graded according to the appearance of the blastomeres and the percentage of fragments, using conventional criteria[18]. Cleavage embryos were considered high quality if they met the following criteria: i) their fertilization was normal; ii) they had at least six blastomeres, iii) the blastomere size was stage-specific, iv) the percentage of embryo fragments did not exceed 10%; v) the blastomere was transparent and without cytoplasmic inclusions or vacuoles; and vi) there were no multinucleated blastomeres. Suboptimal day 3 embryos were placed in a culture for an extended period to develop to the blastocyst stage. The day 5 embryo quality assessment was based on the scoring system of Gardner and Schoolcraft[19], with embryos graded ≥ 3 BB considered to be good blastocysts. No more than two embryos were transferred in the FET cycles. All embryos were thawed on the day of transfer and post-thaw embryos with at least half of their blastomeres intact were considered to have survived.

Outcome Parameters and Statistical Methods

The study's primary outcome was the live birth rate per ET. The secondary endpoints included the clinical pregnancy, miscarriage, implantation and heterotopic pregnancy rates. A live birth was defined as the delivery of a neonate at or after 24 weeks of gestation. A clinical pregnancy was defined by the presence of at least one intrauterine gestation sac 28 days after the ET. Miscarriage was defined as a pregnancy loss before the 24th gestational week, whereas early miscarriage was defined as a pregnancy loss before the 12th gestational week.

Continuous data were analyzed using Student's *t*-test, and categorical variables were analyzed using the χ^2 test. Multivariable logistic regression was used to evaluate the possible relationships between the types of endometrial preparation and pregnancy outcomes after adjusting for confounding factors, including age at ET, BMI, infertility duration, cause of infertility, duration of cryopreservation, comorbidities, uterine malformation, serum progesterone level on the day before transplantation, endometrial thickness on the ET day, high-quality embryo transfer, number of embryos transferred and stage of embryo development. All statistical analyses were performed using SPSS version 21.0. A *P*-value < .05 was considered statistically significant.

Results

Study Population

Data from the 6,159 patients who fulfilled the inclusion criteria were analyzed, with no loss to follow-up. Among them, 4,306 women underwent NC treatment, 1,007 received HRT and 846 received GnRH-a + HRT.

Baseline Characteristics

Patients' baseline characteristics are presented in Table 1. No significant differences in age of oocyte retrieval or age at ET among the three treatment groups were observed. Due to the study's large sample size, smaller differences between the participants at baseline may have led to statistically significant group differences in body mass index, infertility duration, cause of infertility and duration of cryopreservation. The proportions of polycystic ovary syndrome (PCOS) and endometriosis (EMS) that contributed to infertility in women who received ART were significantly higher in the GnRH-a + HRT-group than the NC- or

HRT-group. The percentage of complications caused by uterine malformation was not statistically significant.

Cycle Characteristics of the FET

As presented in Table 2, the proportion of the day 3 embryos that were transferred was significantly lower in the HRT-group than the NC-group, while the proportion of the day 5 embryos that were transferred was significantly higher in the HRT-group than the NC-group. The distribution of the best embryos transferred was significantly higher in the NC-group than the HRT-group. Endometrial thickness on the day of ET was significantly greater in the NC-group than the other two groups. Serum progesterone levels on the day before transplantation were comparable among the three study groups. The embryo survival rate after thawing and the number of embryos transferred were also similar across the study groups.

Reproductive Outcomes

Reproductive outcomes are shown in Table 3. The live birth rates per ET were comparable in the three groups. The early miscarriage rate and miscarriage rate of singleton pregnancies were significantly higher in the HRT-group compared with the NC-group. The rates of clinical pregnancy, implantation and heterotopic pregnancy were similar among the three groups. The rates of miscarriage did not differ significantly by group, but the early miscarriage rate was somewhat lower in the GnRH-a+ HRT-group.

After adjustment for the above-mentioned confounding factors (Table 4), the early miscarriage rate and miscarriage rate of singleton pregnancies remained higher in the HRT-group compared to the NC-group.

Discussion

Main findings

The rate of early miscarriage of pregnancy per embryo transfer (ET) was higher in the HRT-group than the natural cycle (NC) group (24.2% versus 18.7%, respectively). The unadjusted odds of the miscarriage rate of singleton pregnancies were also significantly higher in the HRT-group compared with the NC-group (25.5% versus 20.4%, respectively). After adjusting for possible confounding factors, the early miscarriage rate and the miscarriage rate of singleton pregnancies remained significantly higher in the HRT-group than the NC-group. The clinical pregnancy rates in the NC-, HRT- and GnRH-a + HRT-groups of women with a history of CS was 48.8%, 48% and 47.1%, respectively; the heterotopic pregnancy rate was 1.33%, 2.30% and 1.25%, respectively, and the live birth rates were 37%, 34.1% and 35.7%, respectively. These findings imply that HRT may increase the risk of early miscarriage during FET cycles in patients with a history of CS, although the rates of clinical pregnancy, implantation and live births were similar.

Strengths and Limitations

This study has strengths: primarily its large cohort size, which is the largest in this area to date. The study provides new insights into current patterns of practice and associated clinical outcomes; moreover, it is the first to investigate the efficacy of different endometrial preparation protocols used for FETs in patients with a CS history. The limitations of this study include its retrospective design. Furthermore, the detailed ultrasound information on uterine myometrial defects, complications related to CS and the number of previous CSs were unavailable in our study. Another limitation is that pregnancy-related complications and

neonatal outcomes were not analyzed, as this information was collected during a telephone follow up and could not be verified for analysis. Further studies and randomized controlled trials are required to document the complications of CS and compare the maternal and neonatal safety of the protocols examined in this study.

Interpretation

The short- and long-term complications of CS are infection, increased hemorrhage risk, reduced fertility and increased risk of obstetric complications in subsequent pregnancies (placental abnormalities, caesarean scar pregnancies and uterine rupture)[20, 21]. Given these risks, studies have examined the impact of previous CS on infertility and reproductive outcomes (rates of reduced pregnancy, live births, transplantation and early miscarriage) in IVF/ICSI cycles[11-13]. However, even in the "in phase" endometrium, the supraphysiological steroid levels achieved with COS may negatively affect endometrial receptivity[22, 23]. The risk of developing intracavitary fluid during hormonal stimulation for IVF was almost 40% in patients with an existing isthmocele after a previous CS delivery[14]. Theoretically, the accumulation of fluid and mucus may facilitate bacterial growth, reducing the chances of successful IVF[24]. To some extent, FET provides better endometrial receptivity by avoidance of supraphysiological steroid levels and adverse effects of COS[25, 26]. Therefore, it is necessary for secondary infertility patients with a history of CS to transfer frozen embryo. Many women with a history of CS by ART have been in need of FET for their second progeny since the release of the second-child policy. Given the importance of endometrial preparation for FET success, physicians should improve their understanding of the effects of different protocols on pregnancy outcomes in patients with a CS history.

Our results are consistent with those of a retrospective cohort study that found early miscarriage after FET in women with previous CS was 20.7%[27]. These results were supported by Naji's research[28]. These studies suggest an positive association between a scarred uterus and a higher spontaneous miscarriage rate. There is increasing evidence that a large number of early miscarriages are caused by impaired decidualization[29]. It is possible that the presence of CS scarring further aggravates this process. Two studies reported that a history of CS leads to a defect in the anterior lower segment of the uterus in 42%-58% of women[30, 31]. Another study found fewer leukocytes and less vascularization at the scar site than in the endometrium of women with an unscarred uterus[32]. A delay in endometrial maturation, which was also found at the scar site, was caused by disruption in steroid receptor expression. However, this total was obtained by summing the results of all the endothelial preparation protocols.

Based on previous researches, we also investigate the efficacies of three cycle regimens in women receiving frozen embryo transfer (FET) with a history of CS, which found that hormone replacement therapy for endometrial preparation further increases the risk of early miscarriage in patients with history of CS. Embryos are transferred to the endometrium prepared by either normal ovulation or hormonal replacement with or without a gonadotrophin releasing hormone agonist. According to some studies, early miscarriage rates are higher with HRT-endometrial preparation for FET than other protocols, even though the pregnancy rates were similar[15, 33]. The reason for the higher miscarriage rate seen in HRT cycles remains unclear. Other research suggests high miscarriage rates are related to PCOS, high and low body mass index[34], an environment with excessive estrogen or a suboptimal ratio between progesterone and estradiol[35]. However, the proportion of PCOS was higher in

the HRT + GnRh-a-group than the other two groups in the present study. The regression analysis in the present study that adjusted for important confounders revealed the risk of early miscarriage was significantly higher in women with a history of CS using the HRT protocol than in the women using the NC protocol. Thus, age at the time of ET, body mass index, cause of infertility, duration of cryopreservation, proportion of PCOS, hormone level before ET, developmental stage at ET, endometrial thickness on the day of ET and the number/percentage of quality embryos transferred did not play an independent role in early miscarriage after FET of women with history of CS. Interestingly, our study also found that the clinical pregnancy and live birth rates in the HRT + GnRh-a-group were similar to the rates of the other two groups, whereas the miscarriage and early miscarriage rates were lower than the other two groups, although not significantly lower. This finding may be related to the role of GnRH. Research has found GnRH expression in the endometrium can directly inhibit inflammatory factors and increase endometrial adhesion molecules.[36, 37] Indeed, two retrospective studies[38, 39] have reported benefits of GnRH-a pretreatment on pregnancy outcomes following artificial-cycle frozen-thawed embryo transfers, including improved clinical pregnancy rates and lower pregnancy loss rates. Given the high risk of CS-related miscarriages, we hypothesize that exogenous estrogen and progesterone could not improve the endometrial environment and that decidualization was impaired and steroid receptor expression disrupted by the presence of a CS scar resulting in later implantation within the window or an implantation site closed to the scar. Therefore, the HRT protocol should be avoided in the FET cycle for women with a history of CS.

Conclusion

In women undergoing FET with a history of CS, HRT for endometrial preparation was associated with a higher early miscarriage rate, albeit after statistical adjustment for confounding factors. This study could help physicians improve their understanding of the impact of different protocols on pregnancy outcomes in patients with a history of CS. Prospective randomized trials should be conducted to assess the efficacy of endometrial preparation protocols for FET in women with a history of CS.

Contribution to authorship

Conceived and designed the experiments: G.F L.G and L.GX. Performed the experiments: G.RX. Organized the data: L.Y and S.J. Contributed reagents/materials/analysis tools: W.Q. Wrote the manuscript: G.RX.

Disclosure of interest

None declared.

Details of ethics approval

Institutional ethic committee approval was obtained on 30 July 2021 by the Institutional Ethics Committee of the Central South University, Changsha, China (2021-KT50).

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