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Effect of intrauterine injection of human chorionic gonadotropin before frozen–thawed embryo transfer on pregnancy outcomes in women with endometriosis

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Abstract

Objective: To investigate the effect of human chorionic gonadotropin (hCG) intrauterine injection before frozen–thawed embryo transfer (FET) in women with endometriosis.

Methods: This retrospective cohort study included 45 women with endometriosis who underwent hCG intrauterine injection before FET; each woman was matched with three patients with endometriosis who did not receive hCG intrauterine injection (controls). Data on pregnancy and prenatal outcomes were extracted from medical records and compared.

Results: Patients in the hCG intrauterine injection group had significantly higher rates of pregnancy and clinical pregnancy (64.4% and 57.8%, respectively) than controls (47.4% and 39.3%, respectively). Neonatal birth weight for both singletons and twins was significantly higher in the hCG group (3486 ± 458 g and 2710 ± 437 g, respectively) than in the control group (3195 ± 401 g and 2419 ± 370 g, respectively).

Conclusion: Pregnancy rate, clinical pregnancy rate, and birth weight were improved in women with endometriosis who underwent intrauterine hCG injection compared with those who did not receive hCG before FET.

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Introduction

Endometriosis (EM) is a chronic gynecological disorder that affects 7% to 10% of women of reproductive age.[1,2] The prevalence of EM increases to 47% in infertile women.[3] Evidence suggests that EM affects *in vitro* fertilization (IVF) outcomes through several mechanisms, including distorted pelvic anatomy, decreased oocyte quality, and impaired endometrial receptivity.[2,4] The endometrium is dysfunctional in women with EM because endometrial receptivity biomarkers such as integrins, osteopontin, leukemia inhibitory factor (LIF), and homeo box A10 (HOXA10) are aberrantly expressed.[5] Recent findings suggest that alteration in progesterone receptor expression, progesterone resistance, and stromal decidualization deficiency are also associated with implantation failure in women with EM.[6,7]

Human chorionic gonadotropin (hCG), as an early embryo-derived signal, initiates trophoblast invasion and regulates implantation. Intrauterine administration of hCG provokes a significant inhibition of intrauterine insulin-like growth factor binding protein 1 (IGFBP-1) and macrophage colony-stimulating factor (M-CSF), whereas LIF, vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP)-9 are significantly upregulated.[8] The latest studies report that intrauterine injection of hCG before embryo transfer (ET) significantly improves pregnancy rates in IVF/intracytoplasmic sperm injection cycles.[9,10] It is suggested that hCG exerts multiple direct influences on the endometrium. However, little is known about the effect of intrauterine hCG infusion on pregnancy outcomes in women with EM. The aim of this study was to investigate the benefits of intrauterine hCG injection in women with EM before frozen–thawed embryo transfer (FET) cycles.

Materials and methods

Subjects

This retrospective cohort study was conducted at the reproductive medicine center of First Affiliated Hospital of Wenzhou Medical University. This study was reviewed and approved by the research ethics committee of our institution (2018-045). All patients in the study provided written informed consent for use of their data.

We analyzed data of women who underwent their first cycle of frozen–thawed embryo transfer from January 2016 to March 2017 at our center. Women with hydrosalpinx, premature ovarian failure, polycystic ovary syndrome, uterine cavity abnormality, recurrent spontaneous abortion, or abnormal parental karyotypes were excluded. Data from 180 women diagnosed with infertility associated with EM were collected, and those who fulfilled the following three criteria were included in the study group: (1) diagnosis of EM confirmed either by laparoscopy and biopsy of the lesion or by ultrasound-guided cyst fluid

drainage pathology examination; (2) age ≤40 years; and (3) underwent hCG intrauterine injection before FET. Forty-five women were enrolled into the study group (hCG group). Women with EM who did not receive intrauterine hCG were matched to the study group as controls at a ratio of 1:3, according to the following criteria: (1) age (±1 year of the study patients matched); (2) type of infertility (primary or secondary); and (3) maximal uterine size within 6 cm in cases of coexistence of adenomyoma or adenomyosis. Pregnancy outcomes were masked during matching.

Endometrial preparation

Hormone replacement therapy was adopted for endometrial preparation. The protocol was started with oral estradiol valerate (Progynova, Schering Pharmaceutical Ltd., Guangzhou, China) following a transvaginal ultrasound scan on cycle day 2 to 5 at a dose of 4 mg/day for 4 days, and then increased to 6 mg/day for 5 days. A second ultrasound scan was performed, serum estradiol and progesterone were checked, and the dose of estradiol valerate was adjusted up to 8 mg/day accordingly. Luteal support was started when endometrial thickness reached 8 mm or more, serum estradiol level ≥600 pmol/L, and progesterone level <5 nmol/L. Progestin (Utrogestan; Besins Manufacturing, Brussels, Belgium) was administered vaginally at 400 mg/day for 3 or 5 days before embryo transfer, depending on the cleavage stage of embryos. Progestin supplementation continued until pregnancy was confirmed at 10 weeks of pregnancy.

Intrauterine hCG injection and embryo transfer

Women in the study group underwent hCG intrauterine injection 1 day before FET. Briefly, the cervical mucous was wiped out using a cotton swab. The insemination cannula was attached to a 1-mL syringe and used in draw up recombinant (r-)hCG (Ovidrel; Merck Serono, Geneva, Switzerland). When the cannula passed through the cervical internal os, 0.04 mL of r-hCG was injected into the uterus.[9,10] High-quality embryos were defined based on the Istanbul consensus as grade 1 or 2.[11] Day 2 to 3 cleavage-stage embryos or blastocysts were transferred. No more than two embryos were replaced in the uterus.

Reproductive outcomes

Pregnancy was confirmed when serum β-hCG was ≥10 IU/L 2 weeks following embryo transfer. Clinical pregnancy was defined when the gestational sac(s) was observed by ultrasonography 4 weeks after embryo transfer. The miscarriage rate per clinical pregnancy was defined as the proportion of patients who failed to progress to 28 gestational weeks in all clinical pregnancies. Biochemical pregnancy was diagnosed if β-hCG decreased before a gestational sac (s) was identified. Total pregnancy loss consisted of biochemical pregnancy and miscarriage. Live birth was defined as the birth of at least one living child.

Statistical analysis

Continuous variables were assessed for normality using the Shapiro–Wilk test and presented as mean ± standard deviations or median (interquartile range) according to normality. Differences between groups were assessed using the *t*-test or Mann–Whitney U-test. Categorical variables were expressed as frequencies and percentages within group. Categorical variables were compared using Pearson's chi-squared or Fisher's exact test, when appropriate. Two-tailed $P < 0.05$ was considered significant.

Results

Eighteen women in the hCG group had 33 fresh embryos transferred and 55 women in the control group had 110 fresh embryos transferred in the ovarian stimulation cycle. All women underwent their first cycle of frozen–thawed embryo transfer. No significant differences in baseline clinical characteristics were detected between the two groups, as shown in Table 1.

Pregnancy outcomes were improved in the hCG group. Patients treated with intrauterine hCG injection had a higher embryo implantation rate, pregnancy rate ($p = 0.048$), clinical pregnancy rate ($p = 0.030$), and live birth rate than their counterparts in the control group, with differences in pregnancy rate and clinical pregnancy rate being significant; details are given in Table 2. No statistical differences were identified with respect to pregnancy loss rate. Gestational age at delivery for singletons and twins was comparable between the two groups; however, significantly lower birth weights for both singletons ($p = 0.032$) and twins ($p = 0.049$) were observed in the control group.

Discussion

In this retrospective cohort study, we aimed to investigate whether intrauterine injection of hCG would benefit patients with EM undergoing FET. The main finding was that the intervention statistically improved pregnancy and prenatal outcomes. Although the increment in live birth rate did not reach statistical significance, patients with EM who received hCG intrauterine injection achieved significantly higher pregnancy rate, clinical pregnancy rate, and neonatal birth weight compared with those who did not receive hCG infusion.

Women with EM have a significant reduction in peak estradiol (E_2) concentration, mean number of oocytes retrieved, fertilization rate, embryo implantation rate, and pregnancy rate during IVF compared with women affected by tubal factor or other infertility diagnoses.[2,4,12] In contrast, other studies have reported comparable pregnancy results between women with and without EM.[13,14] Discrepant conclusions were also found regarding the effect of the severity of EM in assisted reproductive outcomes.[14,15] Herein, we report

Table 1. Baseline characteristics of patients in the hCG infusion and control groups (means ± standard deviation or median and interquartile range).

Variable	hCG group (n = 45)	Control group (n = 135)	*p* value
Age (years)	31.69 ± 4.21	31.73 ± 4.12	0.958
BMI (kg/m^2)	20.41 ± 2.58	20.90 ± 2.50	0.256
Infertility duration (years)	4 (2–5)	3 (2–4)	0.09
Basal E_2 (pmol/L)	130 (94–187)	142 (79–193)	0.909
Basal P (nmol/L)	1.99 ± 0.96	1.85 ± 0.95	0.437
Progestin administration day			
Serum E_2 (pmol/L)	1405 (826–2086)	1190 (814–1902)	0.697
Serum P (nmol/L)	1.92 (1.23–2.97)	1.59 (0.72–2.80)	0.284
En thickness (mm)	10 (8.8–10.6)	9 (8.5–10)	0.203
En thickness on ET day (mm)	11 (9.5–11.7)	10 (9.1–11.3)	0.423
No. of embryos transferred	2 (2–2)	2 (2–2)	0.506
Embryo cultured *in vitro* (days)	3 (3–5)	3 (3–5)	0.846
High-quality embryos transferred	1 (1–2)	1 (1–2)	0.670

BMI, body mass index; E_2, estradiol; P, progesterone; En, endometrium; ET, embryo transfer.

Table 2. Pregnancy outcomes [no./no. (%)] and prenatal outcomes (means ± standard deviation) in the hCG infusion and control groups.

Variable	hCG group	Control group	*p* value
Stage at embryo transfer			0.705
48 hours	1 (2.2)	1 (0.7)	
72 hours	28 (62.2)	87 (64.4)	
Blastocysts	16 (35.6)	47 (34.8)	
Embryo implantation rate	36/82 (43.9)	73/224 (32.6)	0.067
Pregnancy rate	29/45 (64.4)	64/135 (47.4)	0.048
Clinical pregnancy rate	26/45 (57.8)	53/135 (39.3)	0.030
Live birth rate	21/45 (46.7)	43/135 (31.9)	0.072
Total pregnancy loss rate	8/45 (17.8)	21/135 (15.6)	0.725
Biochemical pregnancy rate	3/29 (10.3)	11/65 (16.9)	0.538
Miscarriage rate	5/26 (19.2)	10/53 (18.9)	1.000
Term delivery rate	17/21 (81.0)	32/43 (74.4)	0.755
Singleton delivery rate	16/21 (76.2)	29/43 (67.4)	0.472
GA for singleton (weeks)	39.88 ± 1.23	39.63 ± 1.57	0.582
GA for twins (weeks)	37.37 ± 0.79	36.31 ± 1.53	0.162
Birth weight for singleton (g)	3486 ± 458	3195 ± 401	0.032
Birth weight for twins (g)	2710 ± 437	2419 ± 370	0.049

GA, gestational age.

acceptable pregnancy outcomes in the control group compared with those in the abovementioned studies, with a clinical pregnancy rate of 39.3% and live birth rate of 31.9%.

The effect of EM on IVF outcomes continues to be investigated. Numerous studies have reported impaired endometrial receptivity and decidualization in patients with EM, including attenuated endometrial progesterone response and altered expression of integrins and interleukins, which adversely affect embryo implantation.[5,6,16,17] Interestingly, hCG has been shown to promote human endometrial stromal cell decidualization and lead to a significantly stronger induction of decidualization when used in combination with progesterone.[18,19] Luteinizing hormone (LH)/hCG receptors are expressed in human endometrium on glandular epithelium, mediating the effects of gonadotropins, and hCG acts on the intrauterine environment via LH/hCG receptor;[20,21] therefore, the addition of LH/hCG to endometrium might play a paracrine role in control of endometrium physiology.[22] Transcription of hCG is detected at the two-cell stage[23] in human embryos, and preimplantation communication between hCG and the endometrium is crucial for establishment of pregnancy.[20] Recently, researchers have reported that intramuscular infusion of hCG did not seem to be beneficial for pregnancy outcome in FET[24] but that intrauterine infusion of hCG resulted in higher implantation rate, clinical and ongoing pregnancy rate, and delivery rate.[9,10,25–28] Many of these studies have demonstrated the beneficial effect of intrauterine hCG injection before cleavage-stage embryo transfer, whereas others found no evidence for improvement in blastocyst transfer cycles.[29] The main reason was assumed to be that, with blastocyst transfer, there is not enough time for hCG to have any beneficial effect on the endometrium before implantation, and the effect is both

time- and dose-dependent.[8] On this basis, we planned to overcome this limitation by injecting hCG intrauterine 1 day before FET. However, most previous studies have excluded patients with EM; thus, it was unclear whether intrauterine injection of hCG would benefit these patients. Our results indicated that patients with EM in the hCG infusion group were more likely to achieve a higher pregnancy rate (64.4% versus 47.4%) and clinical pregnancy rate (57.8% versus 39.3%) compared with those in the control group. Although the improvement in the live birth rate (46.7% versus 31.9%) was not statistically significant, this might be because of an insufficient sample size.

We observed that birth weight at delivery was significantly higher in the hCG group for both singletons and twins. Birth weight is affected by numerous factors during pregnancy. Evidence shows that obstetrical complications occurring during the second and third trimesters of pregnancy may originate from local disturbances occurring at the time of implantation.[30,31] Accumulating evidence suggests that the eutopic endometrium of women with EM differs from the endometrium of women without EM, with respect to stem cell content, hormone sensitivity, cellular proliferation, adhesion, invasiveness, angiogenesis, and immune modulation,[32,33] and these abnormalities may lead to impairment in decidualization and placentation in the women affected. hCG forces the formation of villous trophoblast tissue, provokes the development and growth of uterine spiral arteries, and boosts the formation of the umbilical circulation in villous tissue and the formation of the umbilical cord.[34] New data reveal that the LH/hCG receptor is present in fetal organs,[35] and it is thought that hCG promotes organ growth and differentiation in the fetus,[36] and hCG preimplantation signaling modulates immunotolerance and angiogenesis at the maternal–fetus interface.[37] Thus, preimplantation hCG intrauterine infusion might create a fetus-friendly environment that favors early embryo implantation and later fetal growth.

There were some limitations to our study. The retrospective design lowered the power of the conclusion. The sample size of our study was small and hidden biases may be underestimated. Further studies are needed to confirm our findings.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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