Decreased endometrial thickness between HCG day and embryo transfer day improves the pregnancy rate of IVF/ICSI cycles

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Research

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Abstract

Background

Previous studies have suggested that pregnancy outcome and endometrial thickness before embryo transfer are closely related. However, we found that endometrial thickness changed even within a few days between HCG day and embryo transfer day. There have been few studies on the relationship between endometrial thickness change (ETC) and pregnancy outcomes. The aim of this study was to assess whether the ETC from HCG trigger day to embryo transfer day affects pregnancy outcomes of in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles.

Methods

This was a retrospective single-center cohort study. In total, 9773 IVF/ICSI embryo transfer cycles involving the long protocol at Chongqing Reproductive and Genetics Institute from January 1st, 2016, to December 30th, 2019, were included. The 9773 cycles were divided into 2 groups based on whether the endometrial thickness decreased from HCG day to embryo transfer day, and clinical pregnancy rates (CPRs) and ongoing pregnancy rates (OPRs) were compared. Logistic regression was used to determine whether an ETC was an independent risk factor for CPR or OPR.

Results

The CPR and OPR of the decreased endometrial thickness group were higher than those of the non-decreased endometrial thickness group (62.65% and 52.6% vs. 60.56% and 50.18%) (p = 0.041 vs. 0.027). The endometrial thickness change was an independent risk factor for CPR (OR = 0.920, P = 0.003, 95% confidence interval 0.870–0.972) and OPR (OR = 0.907, P = 0.000, 95% confidence interval 0.859–0.958)

Conclusions

A proper decrease in endometrial thickness from HCG trigger day to embryo transfer day in IVF/ICSI cycles increases the CPR and OPR.

Background

Previous studies have suggested that pregnancy outcome is closely related to endometrial thickness. For example, KE Liu reported that the clinical pregnancy rate declines with decreasing endometrial thickness within a range[1]. Therefore, we often measure endometrial thickness by transvaginal ultrasound before embryo transfer. Most doctors choose to cancel an embryo transfer when the endometrial thickness is less than 7 or 8 mm. However, while observing 9773 cycles, we found that endometrial thickness changed in the few days between human chorionic gonadotropin (HCG) day and embryo transfer day. In some

cases, endometrial thickness decreased after the HCG trigger, while in others, it remained the same or continued to thicken. Does this mean that the current embryo transfer cycle should be cancelled for patients with decreasing endometrial thickness? Jokubkiene reported that subendometrial vascularization changes markedly during the normal menstrual cycle. It increases throughout the follicular phase, decreases to a nadir 2 days after ovulation and then increases again during the luteal phase[2]. Does a slight decrease in endometrial thickness during the stimulation cycle imply changes in subendometrial blood vessels, and does this change adversely affect embryo implantation? With these questions in mind, we explored whether the decrease in endometrial thickness from HCG day to embryo transfer day affects pregnancy outcomes.

Materials And Methods

Data extraction and cleaning

Data in this study were from the Clinical Reproductive Medicine Management System in Chongqing Reproductive and Genetics Institute. This retrospective cohort study included 9773 IVF/ICSI embryo transfer cycles carried out with long Gonadotropin releasing hormone agonist (GnRH-a) protocol stimulation at Chongqing Reproductive and Genetics Institute from January 1st, 2016, to December 30th, 2019. The inclusion criteria were as follows: all the cases underwent controlled ovarian stimulation (COS) with the long GnRH-a protocol, and all the embryos were transferred during the cleavage stage. The exclusion criteria were as follows: age over 40 years, progesterone level above 1.5 ng/ml on HCG day, endometrial thickness < 7 mm on embryo transfer day, or no embryo to transfer.

Ovarian stimulation protocol and embryo transfer strategy

Patients undergoing the long protocol were treated with a GnRH-a to downregulate the functions of the pituitary gland on day 21 of the previous menstrual cycle. After downregulation of the pituitary gland, gonadotropin was initiated with a starting dose of recombinant FSH ranging from 112.5 to 300 IU, and gonadotropin was modulated according to the patient’s ovarian response. Recombinant HCG (250 μg, MerckSeronoS.P.A.) was used to trigger ovulation in all cases when at least two mature follicles were observed to reach 18 mm. Three independent doctors specialized in ultrasound studies performed measurements completely independently and were blinded to the pregnancy results. The results of each doctor's examination were checked by the two other doctors, and the coefficient of variation measured by the different doctors was less than 5%. The oocytes were retrieved 34–36 hours after the HCG trigger, and progesterone gel (90 mg, Crinone 8%, Merck Serono) was administered vaginally on the day of egg collection. Embryo transfer was performed 3 days after oocyte retrieval and the usual IVF or ICSI procedure. Endometrial thickness was measured again in the morning on the transfer day. After embryo transfer, vaginal progesterone gel was continued for luteal support. Venous blood-HCG levels were measured 14 days after embryo transplantation. Transvaginal ultrasonography of the uterus was performed 4 weeks after embryo transplantation, and the presence of a gestational sac and fetal

heartbeat was considered a clinical pregnancy; additionally, intrauterine fetal survival at 12 weeks was considered an ongoing pregnancy.

Statistical analysis

The 9773 cycles were divided into two groups based on whether the endometrial thickness decreased: group 1, endometrial thickness on embryo transfer day was lower than that on HCG day (n = 2616) and group 2, endometrial thickness of embryo transfer day was equal to or higher than that on HCG day (n = 7157). The t-test and chi-square test were used to compare the clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR) of Group 1 to those of Group 2. Then, logistic regression analysis was performed to determine whether the ETC was an independent risk factor for pregnancy outcome. SPSS (Statistical Package for Social Science, SPSS Inc, Chicago, IL)) 24.0 was used for analysis. $p < 0.05$ was considered to be statistically significant.

ETC = endometrial thickness on transplant day - endometrial thickness on HCG day

CPR = clinical pregnancy cycles/transplantation cycles

OPR = ongoing pregnancy cycles/transplantation cycles

Results

There were no significant differences in Age or other basic indicators between the two groups. However, the Estradiol level on HCG day and Duration of ovarian stimulation in group 1 were higher than those in group 2, and the Endometrial thickness on HCG day in group 1 was higher. The Endometrial thickness on transplant day in group 1 was lower. It was also observed that the CPR and OPR in group 1 were higher than those in group 2. The difference was statistically significant.

Table 1
Characteristics of basic parameters and cycle parameters of patients in the two groups

	Group 1 (n = 2616) Decreasing group	Group 2 (n = 7157) No decreasing group	P
Age (years)	31.01 ± 3.84	31.17 ± 3.81	0.075
Infertility years (years)	5.27 ± 3.71	5.16 ± 3.66	0.301
BMI	21.96 ± 2.73	21.99 ± 2.79	0.601
Infertility type			
Primary infertility (%)	49.69% (1300/2616)	43.80% (3135/7157)	0.072
Secondary infertility (%)	50.31% (1316/2616)	56.20% (4022/7157)	0.072
Infertility reason			
Tubal factor (%)	80.85% (2115/2616)	82.39% (5897/7157)	0.078
Endometriosis (%)	0.88% (23/2616)	1.09% (78/7157)	0.424
Male factor (%)	8.41% (220/2616)	7.87% (563/7157)	0.404
Multiple factors (%)	2.29% (60/2616)	2.25% (161/7157)	0.958
Unexplained infertility (%)	7.57% (198/2616)	6.40% (458/7157)	0.126
Estradiol on trigger day (pmol/L)	3112.24 ± 1191.99	3047.36 ± 1245.72	0.018*
Duration of ovarian stimulation (days)	10.86 ± 1.33	10.71 ± 1.34	0.000*
Total dose of gonadotropin (IU)	2396.31 ± 762.97	2382.88 ± 744.27	0.433
Number of implanted embryos	1.93 ± 0.25	1.92 ± 0.27	0.089
Endometrial thickness on HCG day (mm)	11.24 ± 1.43	9.66 ± 1.45	0.000*
Endometrial thickness on transfer day (mm)	9.75 ± 1.29	10.62 ± 1.58	0.000*
ETC (mm)	-1.482 ± 0.76	0.963 ± 1.03	0.000*
Number of oocytes retrieved	10.01 ± 3.90	9.88 ± 3.91	0.161
Top-quality embryos	1.77 ± 1.21	1.84 ± 1.20	0.140

Data are presented as n(%), mean ± SD as appropriate

*Statistically significant P-value < 0.05

	Group 1 (n = 2616) Decreasing group	Group 2 (n = 7157) No decreasing group	P
Clinical pregnancy rate (%)	62.65 (1643/2616)	60.56% (4332/7157)	0.041*
Ongoing pregnancy rate (%)	52.60% (1485/2616)	50.18 (3883/7157)	0.027*
Data are presented as n(%), mean ± SD as appropriate			
*Statistically significant P-value < 0.05			

ETC was found to be an independent risk factor for CPR (OR = 0.920, 95% CI 0.870–0.972, p = 0.003) and OPR (OR = 0.907, 95% CI 0.859–0.958, p = 0.000) after adjustments for Age, Infertility type, Infertility reason, Estradiol on trigger day, Duration of ovarian stimulation, Endometrial thickness on HCG trigger day, and Endometrial thickness on transfer day. From the logistic regression equation, we can see that ETC, Age, Infertility type, Duration of ovarian stimulation, Number of implanted embryos, and Endometrial thickness on transfer day were still the factors affecting pregnancy outcome, and endometrial thickness on trigger day and estradiol on trigger day were excluded from the equation (Table 2).

Table 2
The relationship between ETC and pregnancy outcome with logistic regression

	CPR			OPR		
	OR	95% CI	P	OR	95% CI	P
ETC (mm)	0.920	0.870–0.972	0.003	0.907	0.859–0.958	0.000*
Age (years)	0.935	0.922–0.947	0.000	0.920	0.907–0.932	0.000*
Infertility type	1.118	1.005–1.224	0.040	1.184	1.066–1.315	0.002*
Infertility reason	1.012	0.967–1.059	0.596	1.030	0.985–1.077	0.199
Duration of ovarian stimulation (days)	0.949	0.915–0.985	0.006	0.956	0.921–0.992	0.016*
Number of implanted embryos	3.558	2.933–4.316	0.000	3.107	2.549–3.788	0.000*
Endometrial thickness on transfer day (mm)	1.107	1.067–1.148	0.000	1.091	1.052–1.131	0.000*
Data were analyzed by logistic regression using Enter variables. (inclusion variables including ETC; Age; Infertility type; Infertility reason; Duration of ovarian stimulation; Estradiol on trigger day; Number of implanted embryos; Endometrial thickness on HCG day ; Endometrial thickness on transfer day)						
*Statistically significant P-value < 0.05						

Discussion

In this study of 9773 fresh embryo transfer cycles, we found that endometrial thickness after HCG trigger is not invariable; endometrial thickness decreased in 2616 cycles and not decreased in 7157 cycles (including cycles in which endometrial thickness did not change and cycles in which endometrial thickness increased). We found that the pregnancy rates were higher in the decreasing group. ETC was an independent risk factor for CPR and OPR. This conclusion is consistent with that of Jigal Haas's study[3].

L. Jokubkiene studied the changes in endometrial blood flow in 14 volunteers during the natural cycle and found that during the follicular phase, the sub-endometrial blood vessel index increased with follicular growth and that the thickness and volume of the endometrium increased rapidly. The sub-endometrial blood vessel index decreased to the lowest point 2 days after ovulation and then increased again during the luteal phase[2]. This finding seems to explain the decrease in endometrial thickness in some patients during the stimulation cycle between the HCG day and embryo transfer day. We hypothesized that this endometrial change, which is highly similar to that in the natural cycle, might lead to better receptivity of embryos. Sarani SA proposed that 1 to 5 days after ovulation is the period of the

menstrual cycle when endometrial vascularization is at its lowest, and it is also the period when endometrial receptivity is thought to be at its maximum in terms of hypoxia[4]. It has been demonstrated in animal studies that near-atmospheric oxygen concentrations reduce embryo viability, and compromise embryo development and that oxygen tension in the uterus is lowest during the implantation period[5][6]. Popovici RM suggested that endometrial hypoxia stimulates the production of vascular endothelial growth factor (VEGF) in endometrial stromal cells[7]. Tsuzuki T reported that hypoxic stress stimulates the generation of VEGF through hypoxia-inducing factor-1 [8]. The increase in VEGF in turn regulates the angiogenesis of the endometrium and stimulates further growth of the endometrium[9]. In a single-center retrospective control study, Zhiqin Bu found that an increased endometrial thickness after progesterone administration was associated with better pregnancy outcomes for thawed blastocyst cycles [10]. The possible reason for this conflicting result is that all of our research objects were cleavage-stage embryo transfer, with the transfer period being in the early luteal phase, in contrast to that of the blastocyst transfer, which is closer to the middle luteal phase. In addition, the effects of high estrogen levels on the endometrium during stimulation cycles may have led to different results.

We assume that the transformation of the endometrium from the hyperplasia stage to the secretion stage is accompanied by a decrease in vascularization degree and uterine thickness, which leads to a decrease in endometrial oxygen concentration. Hypoxic stress stimulates the expression of VEGF through hypoxia-inducing factor-1, which leads to an increase in the endometrial vascularization degree and further stimulates the endometrium to thicken again. The reason why this change is not obvious on ultrasound is that endometrial thickness in the luteal stage tends to vary within a smaller range. As we found in our study, the endometrial thickness in both groups changed by approximately − 1.482 ± 0.76 and 0.963 ± 1.03 mm, and there was no significant change in endometrial thickness in some cases under ultrasound examination. Even so, different patterns of changes in endometrial thickness within a small range were associated with different pregnancy outcomes.

In addition, in our study, we found that the endometrium continued to thicken in some patients from HCG day to embryo transfer day, which may reflect a relatively deficient progesterone effect, leading to inadequate endometrial densification. According to Usadi et al., this is independent of the actual circulating progesterone concentration; whether serum progesterone levels are normal or abnormally low, secretory endometrial development is similar[11]. Some scholars believe that this may indicate the presence of progesterone receptor defects or endometrial resistance in some infertile women, and related causes include overexpression of BCL-6 and SIRT-1[12], chronic endometrial inflammation, progesterone receptor gene polymorphisms, altered microRNA expression, and epigenetic modifications to progesterone receptors[13][14]. Another theory is that the ratio of estrogen to progesterone plays a role, with too much estrogen causing the endometrium to continue to grow. This conjecture may also explain why the endometrial receptivity of fresh embryo transplantation during the ovulatory cycle is lower than that during freeze-thaw embryo transplantation[15]. This may be because the high estrogen level during the IVF-ET cycle leads to an imbalance in the estrogen-progesterone ratio, which leads to the failure of endometrial compression.

The advantage of this study lies in its large sample size. The measured endometrial thickness on the HCG day represents the end of endometrial hyperplasia. Since this was a retrospective study, we considered the possibility of bias in the measurement of endometrial thickness among different operators, so we had three staff members at our center perform vaginal ultrasounds on the HCG trigger day and embryo transfer day. At the same time, we conducted logistic regression to adjust for a series of confounding factors that may affect pregnancy outcomes, such as age, infertility type, infertility reason, estradiol level on trigger day, duration of ovarian stimulation, endometrial thickness on HCG trigger day, and endometrial thickness on transfer day, so the statistical results were highly reliable. We found that endometrial thickness on the trigger day was out of the equation when factors that may affect pregnancy outcomes were included in the logistic regression equation, which showed that endometrial thickness on the transfer day and ETC were still risk factors for pregnancy outcomes. This is consistent with previous studies showing that endometrial thickness is an important factor affecting pregnancy outcome.

The study's weaknesses include its retrospective nature and the many confounding factors, though we were able to adjust for these factors and analyzed them. In our center, all embryos were frozen when more than 20 oocytes were retrieved or ovarian hyperstimulation syndrome occurred. Since the number of patients with blastocyst transplantation during the egg retrieval cycle is very small in our center, this population could not be analyzed as a single group. We will consider the study of the relevant population in a future experimental design. At the same time, it remains to be seen whether this hypothesis applies to freeze-thaw embryo transfer.

Conclusion

In summary, the endometrial thickness from the HCG trigger day to the embryo transfer day is not uniform in the fresh embryo transfer cycle, and we cannot use decreased endometrial thickness as a basis for cancelling the embryo transfer step in the cycle. In contrast, a decrease in endometrial thickness may indicate a better pregnancy outcome.

Abbreviations

ETC: endometrial thickness change ; HCG:human chorionic gonadotropin; IVF:in vitro fertilization; ICSI:intracytoplasmic sperm injection; CPR:clinical pregnancy rate; OPR:ongoing pregnancy rate; GnRH-a:Gonadotropin releasing hormone agonist; COS:controlled ovarian stimulation; FSH:Follicle-stimulating hormone; VEGF:vascular endothelial growth factor

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Chongqing Maternal and Child Health Hospital. (Approval reference number: 2019LSY1206)

Consent for publication

Written informed consent for publication was obtained from the patients

Availability of supporting data

All data supporting the conclusion of this article are included

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WM contributed to the study design, data analysis and manuscript preparation. Huali. D and Xinlin. W handled patient recruitment. Lihong. Z and FW collected the data. All authors read and approved the final manuscript..

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