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ORIGINAL ARTICLE

Role of Low-dose Human Chorionic Gonadotropin in Follicular Phase for Thin Endometrium in Frozen Embryo Replacement Cycles in *in vitro Fertilization*/ Intracytoplasmic Sperm Injection Patients: A Pilot Study

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ABSTRACT

Introduction: One of the most challenging problems in *in vitro fertilization* (IVF) is patient with thin endometrium. The objective of the study was to ascertain whether daily human chorionic gonadotropin (hCG) for 7 days with estrogen in hormone replacement frozen embryo transfer (FET) cycles during follicular phase can increase the endometrial thickness (ET) and reduce the cancellation of cycles.

Materials and methods: Twenty-five infertile patients with resistant thin endometrium who had antagonist protocol and planned for frozen embryo replacement were recruited. These patients had prior attempts to thicken their endometrium which had failed. All the patients received estrogen daily from D2/3 of cycle. On day 8 or 9 of estrogen administration, 200 IU of hCG was given daily for 7 days. After 7 days on hCG priming (D14/15), ET was measured and progesterone was started accordingly. Identification of an intrauterine gestational sac with fetal heart beat by transvaginal ultrasonography constituted clinical pregnancy.

Results: Mean ET increased significantly from 5.84 to 7.61 mm ($p < 0.01$). About 72% of patients had more than 20% improvement in their ET after hCG priming. About 76% achieved an ET more than 7 mm. Overall, 50% became pregnant. The ongoing pregnancy rate was 40%.

Conclusion: A total of 200 IU hCG endometrial priming for 7 days in the proliferative phase of hormone replacement cycles for FET is a highly promising approach to thicken thin endometrium with failed prior attempts.

Keywords: Human chorionic gonadotropin in *in vitro fertilization*/ intracytoplasmic sperm injection cycles, Human chorionic gonadotropin, Thin endometrium.

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INTRODUCTION

The endometrium is essential for implantation and as such the thickness of the endometrium has been always considered as an index of quality, especially in assisted reproduction where the selected embryos should be transferred ideally in a receptive environment.[1] A thin endometrium is encountered infrequently (2.4%) in assisted reproductive technology cycles. When it does occur, it is a cause for concern as it is associated with lower implantation rate and pregnancy rate. Though pregnancies have been reported at 4 and 5 mm of endometrium, it is apparent that an ET <6 mm is associated with a trend toward lower probability of pregnancy. However, no agreement has been reached on ET, although most clinicians empirically prefer endometrium >7 mm.

Regarding the etiology, it is considered mainly idiopathic; however, a postcurettage surgical complication might be identified in some cases.[2]

Numerous treatments have been tried to improve refractory endometrium, but success has been limited. Current evidence-based medicine has not validated any specific treatment. Regarding improvement of ET and receptivity, in the luteal phase, ever since the early IVF days, drug supplements have been employed in order to increase endometrial receptivity and, thus, to enhance pregnancy achievement.[3] Regarding the proliferative phase, several ways of treatment have been undertaken to circumvent thin endometrium in order to increase ET, but they showed discordant results.

It has been demonstrated that granulocyte colony-stimulating factor (G-CSF) can increase the mesenchymal and hematopoietic stem cells in the bone marrow.[4] The rationale for intrauterine G-CSF instillation is to improve endometrial growth stems from the understanding that

the human endometrium contains a small population of mesenchymal stem-like cells that could be responsible for endometrial cyclical growth and reconstruction. Gleicher et al[5] were the first to report that intrauterine G-CSF instillation improves ET. Subsequently, many studies on intrauterine G-CSF instillation were published with some reporting improvement,[6,7] while others showed no difference.[8,9] The only study that reported a significant increase in implantation rate and pregnancy rate performed FET and had younger patients in their study.[10] Drugs that increase endometrial blood flow have been administered individually or in combination to improve ET. None of these therapies, such as pentoxifylline 800 mg/day and tocopherol 1,000 mg/day given over several months,[11] sildenafil 100 mg/day given as vaginal pessary, L-arginine 6 gm/day,[12] and low-dose aspirin 75 mg/day have met with much success.

In this pilot study, subjects with repeatedly resistant thin endometrium <7 mm were recruited. We sought to investigate the possible role of adding low-dose hCG in the follicular phase, on the endometrial growth and development.

MATERIALS AND METHODS

The study was conducted at the Institute of Reproductive Medicine and Women's Health, The Madras Medical Mission, Chennai, Tamil Nadu, India, after obtaining approval from Institute's Ethical Committee. Twenty-five patients who were planned for fresh donor or frozen embryos were recruited. Informed consent was taken from all the patients.

The inclusion criteria were

- ET below 7 mm, consistently in previous IVF treatments including frozen embryo
- Failure of previous medications (oral estrogens, vaginal sildenafil, vitamin E)
- Fresh donor or frozen embryo replacement cycles
- Hysteroscopy performed with intact endometrial cavity.

All patients were started on estrogen on day 2/3 of their periods. All of them had blood estradiol <50 pg/mL. Estradiol valerate 2 mg thrice daily was given for 5 days followed by 8 mg per day, thereafter. On day 8 or 9 of the cycle along with continuing 8 mg estrogen per day, injections of hCG (200 IU) were given daily for 7 days. After a week on hCG priming (day 14 or 15 of the cycle), ET was measured with transvaginal ultrasound. The hCG priming was discontinued and from the next day progesterone administration was initiated if ET was >7 mm. The luteal phase support consisted of 8 mg of estradiol valerate per day (in four divided doses) along with 800 mg micronized progesterone per vaginally (in two doses) and oral progesterone (Duphaston 10 mg) thrice daily. All ultrasounds were performed by the same operator. All embryo transfers were performed by the same operator. Measurement of the endometrium was performed under high magnification, on the longitudinal plane of the uterus and at the thicker area. Since objectivity of ultrasonic measurement of ET might still be questionable due to intraobserver bias, we calculated more than 10% increase (still subjective) and >20% improvement (more objective) in ET when comparing the results.

Pregnancy test was performed 16 days after progesterone initiation. Clinical pregnancy was considered as presence of gestational sac with fetal heart beat at 7 weeks. Continuous variables were compared using paired sample t-test. Categorical variables were compared using Fisher's exact test. The significance level was set at 5% ($p < 0.05$).

RESULTS

A total of 25 patients were recruited for the present study. The mean age was 38 years. Mean ET in the previous estradiol primed cycle on D8 and D14/15 was 5.49 mm (4.1–6.7 mm) and 5.92 mm (4.6–6.9 mm) (Table 1). In the present cycle, the mean ET at the beginning of hCG treatment (D8) was 5.84 ± 1.30 mm. After hCG follicular priming (on D14/15), the mean ET significantly increased to 7.61 ± 2.62 mm ($p = 0.01$; Table 2). Majority of the patients (88%) experienced more than 10% improvement after hCG priming (Table 2). About 72% of the patients had more than 20% improvement after hCG priming (Table 2). Importantly, 19 out of 25 patients (76%) achieved an ET more than 7 mm. Thirteen out of 25 patients (52%) achieved an ET more than 8 mm. Three patients showed no or minimal increase in ET.

Table 1: Impact of hCG follicular priming in patients with thin endometrium (n = 25)

	Before treatment	*After hCG*	*p-value*
Endometrial thickness	5.84 ± 1.30 mm	7.61 ± 2.62 mm	<0.01
Improvement	–	96%	NA
10% improvement	–	88%	NA
20% improvement	–	72%	NA
Pregnancy rate	0	50%	NA

NA: Not applicable

Table 2: Comparison of ET in estradiol primed and hCG primed cycles

	Prev cycle ET D8 (E2 primed)	*Prev cycle day 14/15 ET (E2 primed)*	*Present cycle D8 pre-hCG ET*	*Present cycle D14/15 post-hCG ET*
Mean	5.49	5.92	5.84	7.61
Standard deviation	0.59	0.67	0.65	1.31
Minimum	4.1	4.6	4.4	5.00
Maximum	6.7	6.9	6.9	10.00

Table 3: Specific characteristics of each patient (n = 25)

Subject	*ET before hCG (mm) D8*	*Parity*	*ET after hCG (mm) D14/15*	*Outcome*	*Improvement*	*10%*	*20%*
1	5.6	P0L0	8.4	Ongoing 18 weeks+	Yes	Y	Y
2	6.0	P0L0	8.4	Nonpregnant	Yes	Y	Y
3	5.3	P0L0	7.1	Ongoing	Yes	Y	Y
4	5.6	P0L0	8.3	Nonpregnant	Yes	Y	Y
5	6.8	P0L0	8.0	Nonpregnant	Yes	Y	N
6	6.0	P0L0	7.2	Ongoing	Yes	Y	Y
7	5.1	P0L0	9.0	Nonpregnant	Yes	Y	Y
8	5.5	P0L0	5.1	No ET	No	N	N
9	5.8	P0L0	7.5	Pregnant/aborted 20 weeks	Yes	Y	Y
10	5.9	P0L0	7.4	Nonpregnant	Yes	Y	Y
11	6.9	P0L0	10.0	Ongoing	Yes	Y	Y
12	5.7	P0L0	8.0	Ongoing	Yes	Y	Y
13	5.0	P0L0	5.8	No ET	Yes	Y	N
14	6.0	P0L0	7.9	Nonpregnant	Yes	Y	Y
15	6.2	P0L0	9.0	Pregnant/miscarriage	Yes	Y	Y
16	6.3	P0L0	8.0	Nonpregnant	Yes	Y	Y
17	6.2	P0L0	7.6	Nonpregnant	Yes	Y	Y
18	4.9	P0L0	8.3	Nonpregnant	Yes	Y	Y
19	5.8	P0L0	6.0	No ET	Yes	N	N
20	5.0	P0L0	5.4	No ET	Yes	N	N
21	6.0	P0L0	6.9	Nonpregnant	Yes	Y	N
22	4.4	P0L0	5.0	No ET	Yes	Y	N
23	6.9	P0L0	9.0	Pregnant	Yes	Y	Y
24	6.3	P0L0	8.1	Pregnant	Yes	Y	Y
25	6.8	P0L0	9.0	Pregnant	Yes	Y	Y

Twenty-two patients with ET had improvement >10%, and 18 patients had ET improvement >20%. Twenty patients had embryo transfer and 10 became pregnant and 8 are ongoing pregnancies. The overall clinical pregnancy rate was 50%. Ongoing pregnancy rate was 40%.

One patient had miscarriage at 20 weeks due to cervical incompetence and the other one had first trimester miscarriage. There was no significant difference in the pregnancy rate between the patients who had ET between 7 and 8 mm and greater than 8 mm after hCG administration ($p = 0.41$).

Specific characteristics of each patient are presented in detail in Table 3.

DISCUSSION

The current pilot study was an attempt to improve ET in patients with repeatedly thin endometrium (resistant to previous treatments). All patients had been previously treated with either extended, gradually increasing dose of 17-beta estradiol up to 8 mg/day with vaginal sildenafil or human menopausal gonadotropin priming or vitamin E administration but with no success in terms of improvement in endometrium, thereby leading to cancellation of embryo transfer. Our study showed improvement in ET by 20% in more than 50% of the patients. The reason for hCG priming was based on the fact that hCG/luteinizing hormone receptors are present in endometrium. The expression of functional receptors appears to be cycle-dependent, being present from the proliferative phase, and regulated by changes in the alternative splicing pattern.[13] Early hCG priming has a positive paracrine effect during luteal phase, and this effect is due to the receptivity of the endometrium, regardless of thickness. Trilaminar morphology of endometrium might be more clinically related to the receptivity capacity of the endometrium than the thickness.[14] Zhu et al[15] investigated histological factors related to endometrial receptivity and related them to the pattern of endometrium—trilaminar or homogeneous—in the late follicular phase during natural cycles. They observed that vascular endothelial growth factor (VEGF), integrin alpha and beta levels, as well as fully developed pinopodes were significantly lower in cases with ultrasonographically homogeneous endometrium (not trilaminar) indicating poor receptivity.

Human chorionic gonadotropin, a major embryonic signal, plays a critical role in the initiation and maintenance of pregnancy. To investigate possible direct effects of hCG on endometrial paracrine function in the human female *in vivo*, Licht et al[16] developed an intrauterine microdialysis system that allowed the continuous

sampling from the uterine cavity over time as well as the application of exogenous hCG and the monitoring of the tissue response to this stimulus. The hCG administration during the secretory phase significantly modulated several endometrial paracrine parameters that correlate with endometrial differentiation (insulin-like growth factor-binding protein 1), angiogenesis VEGF, implantation (leukemia inhibitory factor, macrophage colony-stimulating factor), and tissue remodeling (matrix metalloproteinase 9).[16] Similarly, Bourdiec et al[17] investigated whether hCG can modulate endometrial stromal cell (ESC) receptivity to interleukin-1 (IL-1) during the implantation window and have an impact on angiogenesis. Interleukin-1 appears to exert a direct impact on the receptive endometrium and induces major molecular changes that are essential for embryo implantation. The angiogenic activity *in vitro* was studied using human microvascular endothelial cell line, scratch wound assay, and cell proliferation. They observed that hCG induced a dose-dependent change in ESC receptivity to IL-1 by significantly upregulating the functional signaling receptor IL-1R1 and concomitantly downregulating the decoy inhibitory IL-1R2. Basic research findings like the above mentioned support a paracrine hCG action on endometrium. Hence, in patients with resistant thin endometrium, hCG might really prove beneficial toward enhancing the receptivity of a poorly developed endometrium.

A thorough search through the literature revealed that another group carried out a similar study where 17 infertile patients with successive implantation failures and resistant thin endometrium were recruited.[18] On day 8 or 9 of the estrogen administration and continuing on 8 mg estrogen per day, subcutaneous injections of 150 IU hCG were initiated daily for 7 days. After a week on hCG priming (day 14 or 15), ET was measured. Mean ET increased from 5.2 to 6 mm ($p = 0.008$); 35.3% of the patients had more than 20% improvement of their ET after hCG priming. In contrast, 29.4% patients did not show improvement in ET, whereas 17% achieved an ET more than 7 mm. Overall, 41% of them (7/17) finally delivered. Our study showed 72% patients achieving more than 20% increment in ET after hCG administration, with overall pregnancy rate of 50% with ongoing pregnancy rate of 40%. Only three patients (12%) showed no or minimal increase in ET after hCG administration.

Another group carried out a similar study in oocyte recipients but having normal endometrium.[19] They administered much higher dose of hCG (750 IU) every 3 days concomitant to endometrial preparation with estradiol. Sibling oocytes from the same donor were prospectively shared at random among two different recipient groups: Group I where recipients received 750 IU of hCG every 3 days plus estradiol, and group II where recipients received only estradiol. Remarkably, not only ET was significantly lower in group I, but pregnancy rate also was significantly lower in group I as compared with group II (13.6 *vs* 45.4%, $p < 0.05$). Therefore, the study was discontinued prematurely for ethical reasons when 22 cycles were completed. This scenario indicates a possibility that even hCG beyond a certain dose induces deleterious effect on endometrial receptivity.

One of the strong points in our study design was the fact that we considered improvement in ET only when the thickness increased by 20%. Moreover, we included only patients with resistant thin endometrium (failure of previous medications) of <7 mm.

Our study is not without limitations. We did not have a control group which received placebo. We have taken previous cycles as a control where ET did not improve in spite of various modifications (estrogens, aspirin, sildenafil). Apart from giving hCG, we used aspirin and/or sildenafil citrate. It can be argued that these could have influenced the results we obtained, but we believe that these could not have essential impact on our results because our patients already had failure with these interventions.

Thin endometrium is a difficult problem in assisted reproduction and it really creates frustration among both doctors and patients. In our pilot study, we achieved almost 50% pregnancy rate, which indicates the high potential of this protocol.

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