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ASSISTED REPRODUCTION TECHNOLOGIES

"Follicular HCG endometrium priming for IVF patients experiencing resisting thin endometrium. A proof of concept study"

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Abstract
Purpose A thin endometrium is one of the most difficult problems encountered in assisted reproduction every day practice. Whether a daily dose of 150 IU HCG for 7 days concomitant with estrogen administration in estrogen replacement cycles can increase the endometrial thickness and improve pregnancy outcome, was the objective of the current study.
Methods Seventeen infertile patients with successive implantation failures and resisting thin endometrium, being recipients of fresh donor or frozen embryos were recruited. This was a prospective cohort, proof of concept study, NCT01768247. On day-8 or 9 of the estrogen administration, and continuing 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) endometrial thickness was controlled with ultrasound, and progesterone was initiated.
Results Mean endometrial thickness was increased from 5.2 mm to 6 mm (p=0.008). 35.3 % of the patients had more than 20 % improvement of their endometrial thickness after HCG priming. 17 % achieved an endometrial thickness more than 7 mm, and 29.4 % did not improve their thickness at all. Interestingly, from the later two became pregnant. Overall, 41 % of them (7/17) finally delivered.
Conclusions One hundred fifty IU HCG endometrial priming for 7 days in the proliferative phase of estrogen substituted cycles for frozen embryos is highly promising, as not only the thickness of the endometrium improves but also eventually the receptivity appears normalized.

Keywords Thin endometrium · HCG · Frozen cycles · Estrogen replacement · Implantation · Poor prognosis

Capsule HCG endometrial priming in the proliferative phase of estrogen substituted cycles for frozen embryos improve both thickness and reproductive outcome.

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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 [17]), especially in assisted reproduction where the selected embryos should be transferred ideally in a receptive environment. A thin endometrium it has been reported in 5 % of women <40 years of age and in 25 % of 41–45 years old women [18] and it has also been related to poor pregnancy outcome [20]. Regarding the etiology it is considered mainly idiopathic, however, a surgical complication post-curettage might be identified in some cases [19].

Although, the endometrium during IVF treatment is usually thicker than in a natural cycle (due to exposure to supra physiological levels of estradiol and progesterone), implantation rates are still relatively low compared to natural conception cycles, indicating that thickness might be important but does not guarantee implantation. One reason for this discrepancy might be that endometrial histology during IVF has been found far from normal as compared to the histological image of endometrium during natural cycles [3]).

Regarding improvement of endometrial thickness and receptivity, in the luteal phase, even since the early days of IVF, there have been employed additives as progesterone supplementation in order to increase receptivity and thus to enhance pregnancy achievement [10]. Regarding the proliferative phase, several ways of treatment have been undertaken to circumvent thin endometrium trying to increase thickness with questionable results.

Vitamin-E administration has been shown to be able to improve the endometrial response in unexplained infertile women via its likely antioxidant and the anticoagulant effects. It may also modulate the antiestrogenic effect of clomiphene citrate treatment, known factor of a thin endometrium [6]. Sildenafil is another agent used. Check et al., used sildenafil in women failing to attain an 8 mm endometrial thickness, despite an oral graduated E_2 regimen, (either in fresh oocyte retrieval cycle or frozen embryo transfer). Patients were randomly treated again with either graduated oral E_2 or vaginal sildenafil or vaginal E_2 therapy and neither vaginal E_2 nor sildenafil significantly improved endometrial thickness or blood flow in the subsequent frozen ET-cycle [4]. Others attempted to extend the estrogen administration for 14–82 days, in frozen cycles [5]. In the study group, the mean endometrial thickness increased significantly from 6.7 mm during the preceding controlled ovarian hyperstimulation cycles to 8.6 mm after an extended estrogen therapy in frozen transfer cycles (P=0.031). Similarly the pregnancy rate was significantly higher than that of the control group (38.5 % vs. 4.3 %, P=0.016). In In-Vitro Maturation (IVM) cycles with a thin endometrium both low-dose hMG and micronized 17beta-estradiol supplementation significantly improve endometrial thickness [9]. Recently a case-series was reported, wherein successful endometrial expansion to at least minimal thickness of 7 mm was achieved after uterine perfusion with granulocyte colony-stimulating factor (G-CSF) in four patients previously resistant to treatment with estrogen and vasodilators. All four patients (within 48 h from infusion) had embryotransfer (ET), leading to conception in all cases [11].

In this pilot study, subjects with repeatedly resistant thin endometrium, less than 6 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. We constructed this hypothesis based on the fact that LH/HCG receptor is present in endometrium [15] and therefore a positive interaction could be anticipated when HCG is administered in the proliferative phase of endometrial growth. Furthermore, in a previous study, where hMG (well known that renders its LH capacity due to low dose HCG contain) was compared to rec-FSH during ovarian stimulation, endometrium was more likely to be iso-echogenic and hypo-echogenic in the hMG group [1], also anticipating a possible positive role of HCG activity.

Material and methods

Overall 17 patients being recipients of fresh donor or frozen embryos were recruited. The main inclusion criteria were: (a) consistently endometrium below 7 mm in previous IVF treatments including frozen embryo transfers (we included only less than 6 mm, to avoid measurements bias); (b) history of at least two previous implantation failures with top quality embryos (c) failure of pregnancy so ever; (d) failure of previous modifications (vaginal estrogens, sildenafil, vitamin-E) (e) fresh donor or frozen embryos replacement cycles; (f) hysteroscopy performed with intact endometrial cavity. All subjects gave informed consent. The institutional review board approved the study. The study was registered in ClinTrials.gov registry with the identifier: NCT01768247.

The estrogen replacement protocol followed was as it is described below: Irregular cycling women received a depot Triptorelin 3.75 mg injection the preceding cycle around day 21, and started the estrogen replacement cycle after the period and minimum 14 days after the depot injection. Regular cycling women started the estrogens on day-2 of their period. All women in order to start with estrogens should have blood hormonal work-up with Estradiol<80 pg/ml and Progesterone<1.5 ng/ml. 17-beta estradiol starting dose was 4 mg per os for 3 days, then 6 mg for 3 days, and then 8 mg onwards. On day-8 or 9 of the estrogen administration, and continuing 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) endometrial thickness was checked with transvaginal ultrasound, and hormone analysis was performed. HCG priming discontinued and the next day progesterone administration was initiated for one extra day of the age of the transferred embryos (for a day-3 embryo for example, progesterone started 4 days in advance). All embryo transfers were performed by the same two operators as was the case with the ultrasound.

Pregnancy test was performed 14 days after progesterone initiation (positive considered >5mIU/ml), clinical pregnancy was considered as fetal heart beat at 7 weeks, and delivery recorded of course for each patient.

Measurement of the endometrium was performed under high magnification, on the longitudinal plane of the uterus and at the thicker area. Three measurements were taken and the mean was calculated (Medisson-Samsung X8 echo).

Since objectivity of ultrasound measurements of endometrial thickness might be still an issue of intraobserver bias, we suggested a concept of >10 % increase (still subjective) and >20 % improvement (more objective) when comparing the results.

Pregnancy was considered as more than 2 consecutive rising HCG test above 5mIU/ml. A clinical pregnancy was considered a positive heart beat at 7 weeks, and delivery was recorded for each patient being pregnant.

The luteal phase support consisted of 8 mg of valerate 17-b estradiol (in two doses); 600 mcg micronized progesterone (in three doses).

Continuous variables were compared using paired sample t-test or Mann Whitney test, according to the distribution of their values. Categorical variables were compared using pairs of two tailed Fisher's exact tests. The significance level was set at 5 % (p<0.05).

Results

A total of 17 patients participated in the current study. The mean age was 39 years, and all patients were nulliparas (Table 1). The mean endometrial thickness in the preceding treatment was 5.2 mm (range: 3.4 to 6.0) and during the treatment cycle after HCG follicular priming the endometrial thickness significantly increased to 6.0 mm (minimum 4.2 mm to maximum 8.6 mm), $p=0.008$.

The majority of the patients (52.9 %) experienced more than 10 % improvement after HCG priming. Similarly, 35.3 % of the patients had more than 20 % improvement after HCG priming. Importantly, 3 out of 17 patients (17 %) achieved an endometrial thickness more than 7 mm. These three patients conceived. On the contrary, only 29.4 % (5 out of 17) did not manage to increase their endometrium. Interestingly, two out of these five patients that had the same thickness with and without HCG administration, not only got pregnant but also delivered (Table 2). This finding might imply a collateral positive impact of HCG administration irrespective of the ability to increase endometrial thickness.

Regarding the achievement of a pregnancy, more than half of the patients became pregnant and 41 % of them (7/17) finally delivered. One patient had no embryotransfer and one patient had an ectopic pregnancy. As shown in Table 2, from the six patients with improvement>10 %, three became pregnant (50 %), while from the six patients that presented improvement >20 %, four became pregnant (66.6 %). Specific characteristics of each patient are presented in details in Table 2.

Discussion

The current pilot study, attempting to improve endometrial thickness in patients with repeatedly thin endometrium resisting in previous treatments showed that a weekly co-administration of HCG with estradiol improved both thickness and reproductive outcome. All patients had been previously treated with extended and gradually increasing dose of 17-beta estradiol up to 12 mg/day, as well as vaginal administration. Others had received sildenafil, some hMG priming, and some vitamin E administration, without succeeding in pregnancy occurrence. In this pilot study not only succeeded to improve the endometrial thickness by 20 % in more than a third of the patients, but interestingly even the two patients that had no response to the treatment achieved a pregnancy. This indicates a plausible positive paracrine effect of early HCG priming, days later during luteal phase, and related to the receptivity of the endometrium, regardless thickness.

This might be true, as some studies indicate that trilaminar morphology of endometrium might be more clinically related to the receptivity capacity of the endometrium that the thickness itself [7]. Zhu et al. investigated histologically factors related to endometrial receptivity, and related them to the pattern of endometrium -trilaminar or homogeneous- in the late follicular phase during natural cycles [21]. They observed that 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.

The reasoning for HCG priming was based on the fact that HCG/LH receptors are present in endometrium and even 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]. 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 and colleagues [12] developed an intrauterine microdialysis system that allows 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. HCG administration during the secretory phase significantly modulated several endometrial paracrine parameters that correlate to endometrial differentiation (IGFBP-1), angiogenesis (VEGF), implantation (LIF, M-CSF) and tissue remodeling (MMP-9) [12]. Similarly, Bourdiec and colleagues investigated whether HCG can modulate endometrial stromal cell (ESC) receptivity to Interleukin-1 (IL1) during the implantation window and assess the impact on angiogenesis [2]. IL1 appears to exert a direct impact on the receptive endometrium and to induce 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 and they found that HCG induced a dose-dependent imbalance in ESC receptivity to IL1 by significantly up-regulating the functional

Table 1 Endometrium follicular priming HCG impact in patients with thin endometrium ($n=17$)

	Patients before treatment	Patients after HCG priming	p
Age (ys)	39	39	0.9
Parity history	0	0	0.9
Endometrium thickness (mm)	5.18	6.01	0.008
Improvement	–	70.6 % (12/17)	n.a
10 % improvement	–	52.9 % (9/17)	n.a
20 % improvement	–	35.3 % (6/17)	n.a
Pregnancy rate	0	52.9 % (9/17)	n.a
Delivery rate	0	41.2 % (7/17)	n.a

n.a non applicable

Table 2 Certain characteristics of each patient

Subject	Thickness before(mm)	History parity	HCG value	Thickness after (mm)	Outcome after HCG priming	Improvement	>10 % improvement	>20 % improvement
S.H	5,0	Never pregnant	9	6,7	Delivery	yes	no	yes
M. R	5,6	Never pregnant	6	7,1	Delivery	yes	no	yes
Z. H	5,0	Never pregnant	7	5,5	Non-pregnant	yes	yes	no
H. M	5,3	Never pregnant	12	6,5	Delivery	yes	yes	no
S. L	6,0	Never pregnant	5	8,6	Pregnant/Abortion	yes	no	yes
P.M	6,0	Never pregnant	7	6,0	Non-pregnant	no	no	no
S. S	6,0	Never pregnant	9	5,9	Delivery	no	no	no
M. G	6,0	Never pregnant	10	6,0	Non-pregnant	no	no	no
A. I.	5,2	Never pregnant	9	5,5	Non-pregnant	yes	yes	no
P. M	5,6	Never pregnant	5	5,6	Non-pregnant	no	no	no
T. V	5,7	Never pregnant	8	6,1	Ectopic	yes	yes	no
K. N	5,3	Never pregnant	–	5,5	Non-pregnant	yes	yes	no
T. M	6,0	Never pregnant	7	7,2	Delivery	yes	no	yes
P.H	5.5	Never pregnant	–	5.5	Delivery	no	no	no
H.H	3.4	Never pregnant	7	5.0	Non-pregnant	yes	no	yes
H.P	3.1	Never pregnant	–	4.2	no ET	yes	no	yes
A.P	4.5	Never pregnant	–	5.3	Delivery	yes	yes	no

signaling receptor IL1R1 and concomitantly down-regulating the decoy inhibitory IL1R2. Basic research findings like the abovementioned, support a paracrine HCG action on endometrium that especially in poor prognosis patients with resisting thin endometrium might really proved beneficial towards enhancing the receptivity of a poorly developed endometrium.

Searching the literature revealed that another group [14] carried out a similar study in oocyte recipients with normal endometrium, however they administered much higher dose 750 IU of HCG every 3 days concomitant to endometrial preparation with estradiol until HCG injection to the donor. Sibling oocytes from the same donor were prospectively shared at random among two different recipients; in group-1 oocyte recipients received 750 IU of HCG every 3 days plus estradiol, whereas in group-2 recipients received only estradiol. Endometrial thickness was significantly lower in group-1, but in addition, pregnancy rates were significantly lower than in group-2 (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 that possibly there is a dose dependent effect that above a certain threshold induces a deleterious effect on endometrial receptivity.

One of the strong points in the study design was the fact that we considered as really improvement when the thickness increased by 20 % (relative risk of 1.2). Moreover, we included only patients with real thin endometrium, less than 6 mm to reduce the statistical error. We concluded in such thresholds as transvaginal ultrasonography is of limited value as a screening test for abnormal endometrium, if the only parameter of normality is an endometrial thickness of 5 mm or less [16]. Therefore, we tried to minimize the intra-observer bias to minimum as in such low thickness easily mistake can be performed. Nevertheless, the high magnification, the high resolution of the scans and the mean of three measurements eliminates the risk of bias, increasing thus the validity of measurements.

Thin endometrium is a difficult problem in assisted reproduction and as such it really creates frustration to both doctors and patients. To compare our results, there is a retrospective analysis of 35 embryo transfers performed with a maximum

endometrial thickness of <6 mm [8]. There were only three clinical pregnancies (8.5 % per transfer), two live delivered babies (5.7 % pregnancy rates per transfer). One of the live births was a fresh transfer using a minimal stimulation protocol and the endometrial thickness was 5.8 mm and the other a frozen embryo transfer with a maximum thickness of 5.0 mm. In our pilot study we achieved almost 50 % pregnancy rate, which indicates the high potential of this protocol even if thickness is not always improved.

Concluding, 150 IU HCG endometrial priming for 7 days in the proliferative phase of estrogen substituted cycles for frozen embryos is highly promising, as not only the thickness of the endometrium might improves but eventually the receptivity appears almost normal. A large-scale study should be performed urgently to access the validity if our results.

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