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Endometrial morphology and modulation of hormone receptors during ovarian stimulation for assisted reproductive technology cycles

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Abstract

Objective—To study the biochemical and morphologic implications of different hormonal levels on endometrial development during ART cycles.

Design—Prospective controlled study.

Setting—University center.

Patients—Eleven women during a natural (controls) and 11 oocyte donors during an ART cycle (treated).

Interventions—At the time consistent with day 3 embryo transfer, a transvaginal ultrasound, an endometrial biopsy, and blood sampling were performed. Morphology and thickness of the endometrial stripe were recorded. Real-time RT-PCR was used to measure mRNA levels for ER- α , ER- β , PR-A and PR-B in the endometrial tissue.

Main Outcome Measures—To evaluate morphologic and biochemical endometrial development.

Results—Endometrium was mostly trilaminar (proliferative-like pattern) and thicker in the treated group as opposed to homogeneous and thinner in the controls. PR-B mRNA expression increased 41% in treated patients ($p < 0.05$); PR-A mRNA expression, instead, was unchanged. Serum estradiol and progesterone were higher in the treated group than in controls. FSH and LH levels were similar.

Conclusions—When compared with natural cycles, exposure of the endometrium to high hormone levels during ovarian stimulation significantly increased PR-B receptor expression at the time of embryo transfer. Concurrently, a proliferative-like endometrial pattern persisted. These findings reflect a delayed endometrial development in ART.

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Keywords

Endometrial development; ART; hormone receptors; GnRH-antagonist; real time RT-PCR; ultrasound

Introduction

After oocyte fertilization, the endometrium initially provides nourishment to the embryo by means of its secretions, and subsequently becomes the designated site for its implantation and development. It is regulated by the two principal sex hormones, estradiol and progesterone, with modulation of many different genes, which remain incompletely characterized.

In 2008, the Society for Assisted Reproductive Technology (SART) reported that 32.3% (range 16.7–47.3%, depending on the age group) of fresh non-donor transfers and 55% (all ages) of fresh donor oocyte transfers resulted in live births (1). This discrepancy was present even when comparing only fresh, non-donor, transfers in women younger than 35 years (47.3%). It has been postulated that a possible cause for the lower pregnancy rate in non-donor cycles could be endometrial impairment (2–5). Endometrial development has been defined by histological as well as biochemical and gene expression changes (2–5). Exposure to supraphysiologic levels of estradiol would produce an advanced maturation (1,5). In cycles down-regulated with GnRH (Gonadotropin-Releasing Hormone)-antagonists (ganirelix acetate), the advancement is thought to be 1 to 2 days or more (6–7).

In previous studies, oocyte donors represented the population of choice because they undergo ovarian stimulation and oocyte retrieval, but not an embryo transfer (2–7). An endometrial biopsy was performed during late proliferative to mid-luteal phase in all studies. However, different studies addressed different days of the cycle, only one endometrial sampling was performed per patient, and most studies did not report the morphologic appearance and/or thickness of the endometrium at sampling. Finally, most studies did not report the serum levels of the steroid hormones and/or the amount of medications used for the stimulation.

In this study, we hypothesized that the high serum hormonal levels in assisted reproductive technology (ART) cycles would impair estrogen and progesterone receptor expression in the endometrium. We sought to evaluate endometrial development through the morphologic appearance of the endometrium on ultrasound, as well as through quantification of estrogen and progesterone receptors expression during natural and ART cycles. In addition, we correlated these features with the serum levels of the steroid hormones and the duration of GnRH antagonist use.

Materials and methods

All subjects gave written informed consent to participate in the study. Institutional review board approval was obtained prior to initiate the study from the Wayne State University Human investigation Committee. All had regular, 27-to-30-day menstrual cycles. All normal volunteers in the control group used LH surge kits to predict ovulation and underwent ultrasound to confirm ovulation and the appearance of a corpus luteum. Transvaginal ultrasonography was performed every 2–5 days starting on cycle-day 3 until the day of endometrial sampling. On day 3 post-ovulation (LH+5 for natural cycles, hCG+5 for ART cycles) they underwent transvaginal ultrasonography, an endometrial sampling using a flexible pipelle, and blood sampling. The oocyte donors underwent ovarian stimulation with

gonadotropins and ganirelix acetate for pituitary down-regulation (AntagonTM, Organon, West Orange, NJ). They were treated with oral contraceptives for 10–20 days prior to starting ovarian stimulation. Gonadotropins were administered from stimulation day 1 until the day of the hCG (human Chorionic Gonadotropin)-trigger following a step-up protocol. Ganirelix acetate (0.25 mg daily) was added beginning the day when at least one follicle reached 14 mm in diameter and continued until, and including, the day of hCG administration. HCG was administered when at least 2 follicles were greater than 18 mm in diameter. Oocyte retrieval was performed 35–36 hours following hCG administration.

The thickest endometrial segment (between the two interfaces of the endometrial-myometrial junction) was measured transvaginally on a ‘frozen’ midplane, longitudinal section of the uterus by two-dimensional ultrasound. Endometrial morphology was scored as ‘trilaminar’ or ‘homogeneous hyperechoic’ (Figure 1) by 2 different investigators for all the study patients.

Endometrial tissue samples were obtained by multiple strokes (4–5) of a flexible aspiration pipelle (Ref. 1.103.000 [US 8200], Prodimed, France) of the endometrial cavity. The samples were flash-frozen in liquid nitrogen within 15 minutes from the biopsy procedure and were stored at –72°C. All samples were thawed and processed at the same time in the same laboratory (the C.S. Mott Center for Human Development, Wayne State University, Detroit, Michigan). We utilized real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) to measure mRNA levels for estrogen (ER) and progesterone receptors (PR) in the endometrial tissue samples: ER- α , ER- β , PR-A and PR-B. ER- α and ER- β are structurally different and were measured separately. PR-A and PR-B are identical except for an additional 164 amino acids present only in the N-terminus of PR-B. For this reason, PR-A expression was obtained by subtracting individual PR-B levels from the combined PR-A + B levels.

Real-time RT-PCR allows exponential amplification of short mRNA sequences (100–600 bases) after conversion into complementary DNA (cDNA). It measures PCR amplification as it occurs, thus allowing precise quantification of gene expression.

Total RNA was extracted from the tissues with the RNeasy Mini-kit (Qiagen, Valencia, CA) per the manufacturer’s protocol. A 20 μ L cDNA reaction volume was then prepared using the QuantiTect Reverse Transcription Kit (Qiagen). Optimal oligonucleotide primer pairs for real-time RT-PCR amplification of reverse-transcribed cDNA were selected with the aid of the software program Beacon Designer (Premier Biosoft, Palo Alto, CA). Human oligonucleotide primers, which amplify variable portions of the protein coding regions, were used. Sequences of the oligonucleotides used for amplification of the receptors are the following: β -actin, sense GCA TTG TTA CAG GAA GTC, antisense TTA CAT AAT TTA CAC GAA AGC (126 nucleotides); ER- α , sense, TGC TGC TGG CTA CAT CAT C, antisense CAG GAC TCG GTG GAT ATG G (158); ER- β , sense ACA CTG GAG AAG GAA TAA G, antisense GGA CTC AGT AAC TCA AGG (100); PR-A+B, sense AGC CCA CAA TAC AGC TTC GAG, antisense TTT CGA CCT CCA AGG ACC AT (254); PR-B, sense CCT GAA GTT TCG GCC ATA CCT, antisense AGC AGT CCG CTG TCC TTT TCT (269).

Real-time RT-PCR was performed with the QuantiTect SYBR Green® RT-PCR kit (Qiagen) and a Cepheid 1.2f Detection System (Cepheid, Sunnyvale, CA). Each reaction was 25- μ L consisting of 12.5 μ L of 2 X QuantiTect SYBR Green® RT-PCR master mix, 1 μ L of cDNA template, and 0.2 μ mol/L each of target-specific primer that was designed to amplify a part of the gene of interest. To quantify each target transcript, a standard curve was constructed using a tenfold dilution series of β -actin standard plasmid (Invitrogen Corp,

Carlsbad, CA). We used β -actin as house-keeping gene in a previous study on peritoneal fibroblasts (8). However, other studies have reported on the stability of β -actin in endometrial samples (9). The PCR reaction conditions for ER- α , ER- β , PR-A+B and PR-B, were programmed for each primer as follows: an initial cycle was performed at 95°C for 14, 15, 18.3, and 18.3 minutes, respectively, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds at 50, 53, 58, and 60°C, respectively, and then product synthesis at 72°C for 30 seconds. A control containing all the reaction components except for the template was included in all experiments. The amount of mRNA was then normalized to β -actin. This process was repeated 3 times per each receptor in each endometrial sample. The 3 measurements were then averaged in a single value for the analysis.

Serum levels of estradiol, progesterone, FSH, and LH were evaluated for all study patients at the same time from thawed serum samples in a central laboratory (University Women's Care Laboratory, Southfield, Michigan) using Immulite® 1000 Immunoassay System (Siemens).

We performed a sample size calculation based on an alpha value of 0.05, a power of 80%, a standard deviation (SD) of 20%, and a clinically significant difference of 30% progesterone A+B receptors expression between the two groups of patients (oocyte donors and controls). Assuming the same number of patients in the 2 groups, we needed 8 patients in each group. The choice of 30% difference was arbitrary, but based on 2 assumptions: in normal endometria, PR-B decreases during the secretory phase (10); in a previous study, peritoneal fibroblasts were treated with estradiol to mimic ovarian stimulation during ART, and we found a 10% increase in PR-A+B (8).

Since the receptors expression measurements were non-normally distributed, we used non-parametric tests for statistical analysis: Mann-Whitney-U test, Wilcoxon rank-sum test, Fisher exact test, and Spearman correlations (SPSS 18.0 statistical package for Windows). We used 95 percent confidence intervals (95% CI) to define internal estimates associated with each probability.

Results

We prospectively followed 22 fertile, healthy volunteers between the ages of 21 and 39 years (95% CI 26–32 years) throughout a menstrual cycle. Eleven subjects were normally cycling women (control group) and 11 were oocyte donors going through an ovarian stimulation cycle (treated group) for ART. Two women in the control group and 2 in the treated group had an unsuccessful endometrial biopsy. They were excluded from the endometrial receptors analyses, but not from the serum hormone analyses. Table 1 summarizes the ovarian stimulation characteristics of the patients who underwent ART cycles for oocyte donation. The duration of ovarian stimulation was 10.0 ± 1.0 days (95% CI: 9 to 10).

Table 2 compares the characteristics of the two groups of women at the time of endometrial sampling (LH+5 and hCG+5). In both groups, serum levels of FSH, LH, and estradiol, were significantly different on the day of endometrial sampling compared to cycle-day 3. FSH and LH levels showed changes opposite to estradiol levels.

The endometrium was significantly thicker in the treated group (95% CI of the difference: 2.4 to 5.4 mm; $p < 0.05$). Endometrial morphology at the time of biopsy was primarily trilaminar (8/9, 90%) in the treated group, as opposed to consistently homogeneous in the control group (9/9, 100%). However, all but one woman (whose endometrium never showed a trilaminar morphology) in the control group had already gone through a phase of trilaminar endometrial morphology 2–3 days before endometrial sampling.

Despite the significant serum hormonal changes, endometrial tissue did not show significant changes in expression of ER- and ER- in the treated compared to the control group. A trend toward increased expression was identified for both estrogen receptors (30% and 17% increase, respectively): for ER- , 95% CI of the difference was 2.32E+07 to 6.30E+07 mRNA copies/μg RNA (ns), and for ER- , 95% CI of the difference was 7.15E+05 to 1.94E+06 mRNA copies/μg RNA (ns).

Total progesterone receptors A+B mRNA expression significantly increased in the treated compared to the control group with a 95% CI of the difference of 7.71E+07 to 2.09E+08 mRNA copies/μg RNA (p=0.02). PR-A mRNA expression was nil in both groups. PR-B mRNA expression increased by 41% in treated patients with a 95% CI of the difference of 9.60E+07 to 2.61E+08 mRNA copies/μg RNA (p=0.04). Figure 2 shows PR-B and PR-A+B expression.

When the expression of PR-B and PR-A+B were correlated with the endometrial pattern and thickness, no significant relationship was found in either the control or the treated group. PR-A+B expression was negatively correlated with the serum LH levels at the time of sampling. In the treated group, the analysis was also performed with the duration of ovarian stimulation and the duration of ganirelix acetate use: no significant correlation was identified.

Discussion

Exposure of the endometrium to ovarian stimulation with gonadotropins and the GnRH antagonist ganirelix acetate increased the expression of PR-B at the time of embryo transfer. Conversely, the expression of PR-A remained undetectable. ER- and ER- both increased, but this increase was not statistically significant. These changes occurred in the presence of elevated serum levels of both estradiol and progesterone and decreased levels of FSH and LH. The morphologic follicular pattern of the endometrium (trilaminar vs homogeneous hyperechoic) during a stimulated cycle persisted longer than in natural cycles. Persistence of a proliferative-like appearance likely reflects a delayed endometrial development, rather than advanced maturation. These results are consistent with our previous findings of the effect of gonadotropin stimulation on endometrial development (11).

The two isoforms of the progesterone receptor, PR-A and PR-B, exhibit distinct biological functions in different organs, but their individual role remains to be fully elucidated. PR-A has, in most cells, inhibitory effects. PR-B has stimulatory effects: it can produce uterine and also mammary hyperplasia (12). In normal endometria, PR-B has been shown to decrease during the secretory phase (10). We found an increased PR-B expression during ovarian stimulation for ART. This represents a probable link between endometrial biochemistry and its morphologic changes: an increased PR-B expression induced by ovarian stimulation would lead to the persistence of a proliferative endometrium. The delayed endometrial maturation would thus be desynchronized with the stage of embryo development at the time of a day-3 transfer, possibly leading to decreased implantation rates in ART cycles.

Our outcome differs from that of previous studies (2–7,13). Timing of endometrial sampling in the current study was on the day of the hypothetical day-3 embryo transfer, whereas in other studies was anywhere from late proliferative to mid-luteal phase. Those studies used Noyes' histological criteria to estimate the advanced endometrial development: this has been challenged as a dependable method (14–16). Coutifaris et al. found that histological dating was of no value in discriminating fertile from infertile women (14).

Horcajadas et al. studied 4 groups of patients to compare endometrial development by histology, immunohistology, microarray genomics, quantitative PCR, and ultrasound (16).

They found a slightly delayed, as opposed to advanced, endometrium in patients treated with GnRH-antagonist when compared to naturally cycling women. The authors implied the non accuracy of histological criteria for dating, but could not extrapolate a clear cut conclusion about the efficacy of using ultrasonography to evaluate endometrial development. In this regards, they failed to evaluate the morphologic changes in the naturally cycling women, thus precluding their ability to make the observations described in our report. The same authors used gene expression pattern analysis to profile endometria from natural cycles and from oocyte donors (17, 18). They found a minimum of 2-day delay, as opposed to advancement, in the activation and repression of 2 different clusters of genes that regulate the implantation period.

Our study presents some limitations; first, PR-A variances were calculated by individually subtracting PR-B from PR-A+B; second, a specific impact on endometrial development of individual medications could not be assessed; third, assessment of endometrial development beyond LH+5 was not performed, thus precluding insight on the day of implantation.

Can ultrasound imaging add more information about endometrial development and 'receptivity' when compared to serum hormone levels, hormone receptor expression, or microarray techniques? Although a trilaminar pattern of the endometrium at the time of oocyte retrieval or embryo transfer has been correlated with higher implantation and pregnancy rates, the value of such an assessment has been called into doubt by conflicting results from different studies (11,19–21). In this study, all patients went through a phase of trilaminar pattern, but natural cycling women evolved to a homogeneous hyperechoic pattern soon after ovulation, whereas ART women had a persistence of trilaminar pattern through the early secretory phase, at the time of a day-three embryo transfer (hCG+5). If we accept the microarray technique and the endometrial morphology suggestion of a delayed endometrial development in ART cycles, then our real-time RT-PCR results would be consistent with the molecular changes resulting from the altered gene expression. This constitutes an important observation of our study, which has not been identified before. Indeed, we confirmed significantly lower LH levels during the luteal phase of ART cycles than in natural cycles (22), and altered endometrial PR-B expression that has been described in infertile women (23).

In conclusion, our biochemical and ultrasound findings characterized the suboptimal endometrial development achieved in ART cycles.

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References

1. Society for Assisted Reproductive Technology (SART) National Summary. 2008www.sart.org
2. Garcia JE, Acosta AA, Hsiu JG, Jones HW Jr. Advanced endometrial maturation after ovulation induction with human menopausal gonadotropin/human chorionic gonadotropin for in vitro fertilization. *Fertil Steril*. 1984; 41:31–35. [PubMed: 6420202]
3. Kolb BA, Najmabadi S, Paulson RJ. Ultrastructural characteristics of the luteal phase endometrium in patients undergoing controlled ovarian hyperstimulation. *Fertil Steril*. 1997; 67:625–630. [PubMed: 9093184]

4. Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smits J, Van Steirteghem A, et al. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. *Fertil Steril.* 2002; 78:1025–1029. [PubMed: 12413988]
5. Mirkin S, Nikas G, Hsiu JG, Díaz J, Oehninger S. Gene expression profiles and structural/functional features of the peri-implantation endometrium in natural and gonadotropin-stimulated cycles. *J Clin Endocrinol Metab.* 2004; 89:5742–5752. [PubMed: 15531538]
6. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Contrasting patterns in in vitro fertilization pregnancy rates among fresh autologous, fresh oocyte donor, and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo-endometrium synchrony. *Fertil Steril.* 2008; 89:20–26. [PubMed: 17224151]
7. Papanikolaou EG, Bourgain C, Kolibianakis E, Tournaye H, Devroey P. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. *Hum Reprod.* 2005; 20:1541–1547. [PubMed: 15705618]
8. Detti L, Saed G, Jiang Z, Diamond MP. Differential expression of estrogen, progesterone, androgen, and prolactin receptors in *in vitro* human fibroblasts isolated from normal peritoneum and adhesions. *J Assist Reprod Genet.* 2008; 25:245–250. [PubMed: 18551363]
9. Bersinger NA, Wunder DM, Birkhäuser MH, Mueller MD. Gene expression in cultured endometrium from women with different outcomes following IVF. *Mol Hum Reprod.* 2008; 14:475–484. [PubMed: 18539642]
10. Mylonas I, Jeschke U, Shabani N, Kuhn C, Balle A, Kriegel S, et al. Immunohistochemical analysis of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in normal human endometrium. *Acta Histochem.* 2004; 106:245–252. [PubMed: 15186931]
11. Detti L, Yelian FD, Kruger ML, Diamond MP, Puscheck EE. Endometrial thickness dynamics and morphologic characteristics during pituitary downregulation with antagonists in assisted reproductive technology cycles. *J Ultrasound Med.* 2008; 27:1591–1596. [PubMed: 18946098]
12. Giangrande PH, Kimbrel EA, Edwards DP, McDonnell DP. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol.* 2000; 20:3102–3115. [PubMed: 10757795]
13. Van Vaerenbergh I, Van Lommel L, Ghislain V, In't Veld P, Schuit F, Fatemi HM, et al. In GnRH antagonist/rec-FSH stimulated cycles, advanced endometrial maturation on the day of oocyte retrieval correlates with altered gene expression. *Hum Reprod.* 2009; 24:1085–1091. [PubMed: 19176542]
14. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril.* 1950; 1:3–25.
15. Coutifaris C, Myers ER, Guzick DS, Diamond MP, Carson SA, Legro RS, et al. NICHD National Cooperative Reproductive Medicine Network. Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertil Steril.* 2004; 82:1264–1272. [PubMed: 15533340]
16. Simon C, Oberyé J, Bellver J, Vidal C, Bosch E, Horcajadas JA, et al. Similar endometrial development in oocyte donors treated with either high- or standard-dose GnRH antagonist compared to treatment with a GnRH agonist or in natural cycles. *Hum Reprod.* 2005; 20:3318–3327. [PubMed: 16085660]
17. Horcajadas JA, Riesewijk A, Polman J, van Os R, Pellicer A, Mosselman S, et al. Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles. *Mol Hum Reprod.* 2005; 11:195–205. [PubMed: 15695772]
18. Horcajadas JA, Mínguez P, Dopazo J, Esteban FJ, Domínguez F, Giudice LC, et al. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. *J Clin Endocrinol Metab.* 2008; 93:4500–4510. [PubMed: 18697870]
19. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). *J In Vitro Fert Embryo Transf.* 1990; 7:146–152.
20. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review. *Hum Reprod Update.* 1996; 2:323–335. [PubMed: 9080229]

21. Sharara FI, Lim J, McClamrock HD. Endometrial pattern on the day of oocyte retrieval is more predictive of implantation success than the pattern or thickness on the day of hCG administration. *J Assist Reprod Genet.* 1999; 16:523–528. [PubMed: 10575580]
22. Tavaniotou A, Devroey P. Luteal hormonal profile of oocyte donors stimulated with a GnRH antagonist compared with natural cycles. *Reprod Biomed Online.* 2006; 13:326–330. [PubMed: 16984758]
23. Armant DR, Jessmon PA, Kruger M, Diamond MP, Coutifaris C, Leach RE. High throughput, cell type-specific analysis of key proteins in human endometrial biopsies links infertility to leukemia inhibitory factor, progesterone receptor-B and integrin α IIb dysregulation. *Fertil Steril.* 2008; 90:S162.

Figure 1.
Endometrial morphology on transvaginal ultrasonography: trilaminar (A) and homogeneous hyperechoic (B).

Figure 2.
PR-B and total PR-A+B mRNA levels in human endometrium at the time of hypothetical embryo transfer in ovarian stimulation cycles (treated) and in controls (* = $p < 0.05$).

Table 2

Ovarian stimulation characteristics of the oocyte donors.

Parameter	Mean±SD	95% CI
Total FSH dose (IU)	1732±790	1178 to 2285
Total LH-like dose (IU)	595±427	297 to 894
Duration of ovarian stimulation (days)	10±1.0	9.1 to 10.3
Duration of GnRH-antagonist use (days)	4.3±1.2	3.5 to 5.2
Start day of GnRH-antagonist	6±1	6 to 7
No. oocytes retrieved	18±4	14 to 21

SD= standard deviation; 95% CI= 95% confidence interval

Table 3

Characteristics of the two groups of women on cycle-day 3 and at the time of endometrial sampling (LH+5 and hCG+5).

Parameter	Normal volunteers (control group)	Oocyte donors (treated group)	95% CI of the difference	p-value
	Mean±SD	Mean±SD		
Age (years)	31.8±5.8	26.2±4.5	2.9 to 8.2	<0.05
Cycle day at EM sampling	18±1	15.0±1.0	2.1 to 3.7	<0.05
Cycle-day 3 serum FSH (mIU/mL)	7.5±3.5	6.1±2.3	0.1 to 2.7	ns
Cycle-day 3 serum LH (mIU/mL)	6.5±2.8	5.0±1.6	-2.6 to -0.5	ns
Cycle-day 3 serum estradiol (pg/mL)	33.0±14.5	36.6±10.2	-1.7 to 8.8	ns
Cycle-day 3 serum progesterone	0.9±0.2	n/a	n/a	n/a
Serum FSH (mIU/mL)	4.2±1.8	1.6±0.6	-3.4 to -1.7	<0.05
Serum LH (mIU/mL)	3.1±2.5	0.4±0.5	-3.7 to -1.6	<0.05
Serum estradiol (pg/mL)	99.1±33.2	1801±856	1,235 to 2,169	<0.05
Serum progesterone (ng/mL)	8.5±4.7	107.5±42.2	72.9 to 125.2	<0.05
EM stripe thickness (mm)	8.5±1.9	12.4±3.3	2.4 to 5.4	<0.05
EM morphology (tril./hom.)	0/9	8/1	-	<0.05

[Mann-Whitney-U and Fisher exact tests]

SD= standard deviation; 95% CI= 95% confidence interval; EM= endometrial; tril.=trilaminar; hom.= homogeneous hyperechoic; ns= not significant; n/a= not applicable.