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Correlation between endometrial dating of luteal phase days 6 and 10 of the same menstrual cycle

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Context: Endometrial maturation, important in the diagnosis of infertile couples, has been evaluated since 1950 using the Noyes criteria. Nevertheless, there is no consensus regarding the most suitable period of the luteal phase for performing the biopsy. **Objective:** This study evaluated the correlation between the histological dating of two endometrial biopsies performed in the same menstrual cycle, on luteal phase days six and ten. **Design:** Prospective study. **Setting:** Human Reproduction Division of the Federal University of São Paulo, referral center. **Patients:** Twenty-five women complaining of infertility had their menstrual cycles monitored by ultrasound and LH plasma levels, to obtain evidence of ovulation. **Procedures:** Endometrial biopsies were performed on luteal phase days LH+6 and LH+10 (luteal phase day 1 = LH+1 = the day that follows LH peak). Dating was done according to morphometric criteria, in which an endometrium sample is considered out of phase if the minimum maturation delay is one day. On day LH+6, blood was drawn for plasma progesterone level determination. **Results:** All patients had an ovulatory cycle (mean LH peak: 47.4 U/L; mean follicular diameter on LH peak day: 18.9 mm; mean endometrial thickness on LH peak day: 10.3 mm; mean plasma progesterone level on day LH+6: 14.4 ng/ml). 14 patients had both biopsies in phase; 5 patients had out of phase biopsies only on day LH+6; 3 had out of phase biopsies only on day LH+10 and 3 patients had out of phase biopsies on both days. McNemar's test showed no statistical difference between these data ($p > 33.36\%$). **Conclusions:** The correlation found between the endometrial datings suggests that biopsies performed on either of these two days are suitable for evaluation of endometrial maturation.

UNITERMS: Endometrium anatomy and histology. Luteal phase. Female infertility.

INTRODUCTION

Evaluation of the luteal phase of regularly cycling women complaining of infertility is directed towards the evaluation of corpus luteum activity and the action of progesterone on the endometrium.

Endometrial maturation, whose role in human reproduction was first recognized by Jones,⁽¹⁾ is evaluated by the Noyes criteria.⁽²⁾ However, new progress in reproductive physiology has raised controversies about these criteria, related to the chronological reference for sampling,⁽³⁾ the most suitable region of the uterine wall for biopsy⁽⁴⁾ and the histological analysis itself, whose subjectivity may cause enough interobserver variation to change the clinical procedures.⁽⁵⁾ Moreover, there is no absolute agreement about the luteal phase period most appropriate for performing the biopsy.⁽⁶⁾

This study evaluated the correlation between the histological dating of two endometrial samples, obtained by biopsies performed on luteal phase days 6 and 10 of the same menstrual cycle.

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METHODS

Twenty five regularly cycling healthy women, complaining of infertility for at least one year, voluntarily agreed to participate in the study group and gave their informed written consent. All procedures were also approved by the Medical Ethics Committee of the Universidade Federal de São Paulo.

Blood samples were drawn from patients between days one and five of the menstrual cycle, for basal plasma levels of LH, FSH and prolactin, measured by immunofluorimetry (normal ranges: FSH: 2.4 to 9.3 U/L; LH: 1.5 to 10 U/L; prolactin up to 14.5 ng/ml). A transvaginal ultrasonograph was also done to evaluate uterine echoes.

From menstrual cycle day nine onwards, follicular diameter⁽⁷⁾ and endometrial thickness⁽⁸⁾ were measured daily by transvaginal ultrasound. When the dominant follicle reached at least 16 mm, daily plasma LH level measurements were started, until an LH peak was demonstrated. The following day was taken as the first of the luteal phase (*LH+1*).

Endometrial biopsies were done on days *LH+6* and *LH+10* with the suction catheter Pipelle. In the first of these, the catheter was introduced with its subterminal orifice directed to the right uterine wall and in the second, to the left.

Histological analyses were done in the portion of the tissue nearest to the embolus. The specimen was fixed in formaldehyde 10% and sections of 4 μ m thickness were stained with hematoxylin-eosin. Endometrial maturity was evaluated through morphometric analysis,⁽⁹⁾ taking into account four semi-quantitative parameters (amount of luminal secretion in the gland, pseudo-stratification of the glandular epithelium, stromal differentiation and edema), estimated on a scale from 0 to 3, and two quantitative parameters (number of supra- and subnuclear vacuoles in the glandular epithelium and the volume fraction of the gland occupied by the epithelium). These analyses were done by the same observer. According to the morphometric criteria, the endometrium was considered as immature (out of phase) if histological analysis showed a maturation delay of one or more days.

On day *LH+6*, blood was drawn for progesterone plasma level determination, which was considered to be normal if a value greater than 7 ng/ml was reached.

In the following cycle, an HSG was performed to evaluate the uterine cavity. Data were analysed by McNemar's test, the significance level being 5%.

RESULTS

The 25 patients had ages between 16 and 38 years (mean 29.6 and standard deviation 5.1 years). The infertility period ranged from 12 to 144 months (mean 57.2 and standard deviation 35 months).

All patients showed ultrasonograph and hormonal evidence of ovulation and Table I shows the parameters of the cycles. The mean LH peak value was 47.3 U/L (range 23 to 91 U/L; standard deviation 17.7 U/L). Mean follicular diameter on day *LH+0* was 18.9 mm (range 14.5 to 24 mm; standard deviation 2.2 mm). Mean endometrial thickness on day *LH+0* was 10.3 mm (range 7 to 13 mm; standard deviation 1.4 mm).

All patients showed a normal uterine cavity on hysterosalpingogram and histological analysis showed no endometrial pathology. According to the dating results, patients could be grouped as shown in Table 2. McNemar's test did not show any statistical difference among these values ($p = 36.33\%$).

DISCUSSION

Inadequate endometrial maturation is associated to many factors.⁽¹⁰⁾ This study excluded patients exhibiting a short luteal phase, low luteal phase progesterone secretion or hyperprolactinemia. Their cycles had follicular and luteal phases of normal length, and pre-ovulatory follicular diameter⁽¹¹⁾ and endometrial thickness⁽¹²⁾ were also normal.

Biopsies were performed on luteal phase days 6 and 10, according to the day of LH peak, which provides the best correlation for histological dating.^(3,9,13) Considering that the endometrial secretory phase is induced by progesterone, and the synthesis of this hormone is LH-dependent, taking the LH peak day as the reference appears to be the most appropriate. The biopsies were done on different uterine walls to minimize the interference of the first one with the second.⁽¹⁴⁾

Eight samples from day *LH+6* and five from day *LH+10* showed maturation delay. As there was no statistical difference between these values, biopsies performed on either of these days seem to be equally effective for determining endometrial maturation.

These results differ from those of Castelbaum et al.,⁽⁶⁾ who did endometrial dating according to the Noyes criteria,⁽²⁾ considering as out-of-phase a delay of 3 days or

Table 1
Parameters of the cycles studied

Parameter	Average \pm SD*	Range
follicular phase length (days)	14.60 \pm 2.21	11 to 20
luteal phase length (days)	13.20 \pm 1.33	12 to 17
cycle length (days)	27.8 \pm 2.35	24 to 33
basal FSH (U/l)	5.30 \pm 1.77	2.53 to 8.93
basal LH (U/l)	6.43 \pm 2.54	1.51 to 9.92
basal prolactin (ng/ml)	8.41 \pm 4.03	1.59 to 14.36
progesterone on day LH+6 (ng/ml)	14.4 \pm 4.5	7.1 to 24.8

*SD = standard-deviation

Table 2
Distribution of endometrial dating on days LH+6 and LH+10

	in phase, day LH+6	out of phase, day LH+6	TOTAL (%)
in phase, day LH+10	14 (56%)	5 (20%)	19 (76%)
out of phase, day LH+10	3 (12%)	3 (12%)	6 (24%)
TOTAL	17 (68%)	8 (32%)	25 (100%)

more. Nevertheless, this apparent disagreement may be partially explained based on methodological differences. Unfortunately, there is no information on luteal phase length, prolactin levels, ovulation confirmation, uterine cavity shape or progesterone plasma levels in that paper. Consequently, these two study groups may be not comparable.

Although endometrial maturity can still be determined via other tests (biochemical, immunohistochemical, electron microscopy, etc), their cost/benefit ratios are prohibitive. Histological analysis remains the most useful method for evaluating endometrial maturation and the morphometric process, despite taking up more time, provides a more accurate result.

RESUMO

Contexto: A verificação da maturidade endometrial, elemento diagnóstico necessário na avaliação do casal com queixa de infertilidade, vem sendo feita desde 1950 através do critério de datação histológica de Noyes. No entanto, não existe um consenso em relação ao período da fase lútea mais adequado para a colheita. **Objetivo:** Avaliar a correlação entre as datações histológicas de duas amostras de endométrio colhidas nos dias 6 e 10 da fase lútea de um mesmo ciclo menstrual. **Local:** Setor de Reprodução Humana da Universidade Federal de São Paulo. **Tipo de estudo:** Estudo prospectivo. Constou da comparação entre duas datações de endométrio num mesmo ciclo menstrual. **Participantes:** 25 pacientes com queixa de infertilidade tiveram um ciclo menstrual monitorizado por ultra-sonografia e medida plasmática de LH, para demonstração de ovulação. **Procedimento:** Biópsias de endométrio foram feitas nos dias LH+6 e LH+10 da fase lútea, considerando-se o dia seguinte ao do pico de LH como LH+1. A datação foi feita de acordo com critério morfométrico, considerando-se o endométrio como fora de fase, se o atraso de maturação mínimo fosse de um dia. No dia LH+6 foi feita dosagem de progesterona plasmática. **Resultados:** Todas as pacientes apresentaram ciclos ovulatórios (média dos valores de pico de LH: 47,3 U/L; média dos diâmetros foliculares no dia do pico de LH: 18,9 mm; média das espessuras do endométrio no dia do pico de LH: 10,3 mm; média das concentrações de progesterona plasmática no dia LH+6: 14,4 ng/ml.). Em 14 pacientes, as duas biópsias estavam em fase. Houve atraso de maturação apenas no dia LH+6 em cinco pacientes; apenas no dia LH+10 em três pacientes e, nos dois dias, em três pacientes. Não houve diferença estatística entre esses valores (teste de McNemar, $p=33,36\%$). **Conclusões:** Os resultados sugerem que a colheita do endométrio em qualquer dos dias (sexto ou décimo) da fase lútea fornece resultados semelhantes em relação à maturidade endometrial.

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