Relationship Between Estradiol 17-β and Endometrial Echotexture During Natural and Hormonally Manipulated Estrus in Mares

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Significant changes in endometrial echotexture occur in the uterine body. The time frames where mean numeric pixel values (NPV) of the endometrium decreased or increased in relation to ovulation were influenced by hormonal treatment. It was concluded that estrus-induction with prostaglandin F2α (PGF2α), combined with human chorionic gonadotropin (hCG), was associated with a shortened duration of estrus and accelerated changes in endometrial mean NPV compared with the natural estrus. Increasing levels of estradiol 17-β were associated with decreasing endometrial mean NPV. Mares showing minimal changes in endometrial echotexture during estrus had lower levels of estradiol 17-β. Estradiol 17-β levels peak and then decline during natural estrus, but in mares treated with hCG, estradiol 17-β levels consistently decline through estrus until ovulation. Authors’ addresses: 1405 105 Street, Edmonton AB T6J 5P3, Canada (Bragg Wever); Department of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, Reproductive Biology Research Unit, Royal University Hospital, University of Saskatchewan, Saskatoon, SK S7N 5B4 (Pierson); and Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada (Card). © 2002 AAEP.

1. Introduction

Mares are unusual in that they have a prolonged period of sexual receptivity, making it difficult to predict ovulation and to ensure that insemination and/or breeding occurs within 24–48 h before ovulation. Because ovulation cannot always be predicted accurately by behavior, multiple breedings and/or inseminations are often used to maximize conception rates in mares. The use of multiple breedings per cycle was a common means to achieve pregnancies when natural breeding or artificial insemination with fresh semen was used on the farm where the stallion resided. The number of mares bred and the distance between stallions and mares has increased with the advent of technology to extend the viability of processed spermatozoa (i.e., cooled, transported semen and cryopreserved semen). Furthermore, the cost and availability of processed semen have become important factors limiting the use of more than one breeding per cycle. To synchronize the mare’s ovulation with semen availability, ovulation induction agents, such as human chorionic gonadotropin (hCG), are commonly used.

Transrectal ultrasonography is now used routinely to assess the reproductive tract of the mare.
The non-invasive nature of ultrasonography allows frequent serial examinations to evaluate morphological changes within the endometrial layer of the uterus. The ultrasonographic appearance of the uterus changes dramatically throughout estrus. The presence of ultrasonographically detectable endometrial edema or edema begins at the onset of estrus. Ultrasonographic images of the estrous endometrium resemble cross-sectional slices of an orange or spokes radiating from a wheel. An interdigitating pattern of alternating anechoic and echoic areas in the endometrium is visualized through ultrasonography. The areas of decreased grey scale values or relatively anechoic areas (deeper shades of grey) correspond to the periphery of the endometrial folds, while more echoic (lighter shades of grey) areas correspond to the core of the edematous portion of the folds where a number of layers are simultaneously imaged. Recently, computer-assisted image analysis is being used in conjunction with routine diagnostic ultrasonography to assess changes in ovarian and uterine tissue. Computer-assisted image analysis permits the assessment of changes in ovarian and uterine echotexture through digital evaluation of the pixels in the image. Echotextural differences can be assessed in each area of interest. Computer-assisted image analysis also is advantageous because of the high degree of precision that can be obtained if properly implemented.

Echotextural changes of the endometrium are largely associated with changes in ovarian steroid hormone levels. Endometrial folding patterns during estrus generally parallel increased estrogen production. Uterine echotexture in diestrus was considered to be homogenous. Response rates to hCG were high in mares that had good endometrial edema. Hormonal agents to induce estrus and ovulation are used widely in clinical practice. The effect of ovulation induction agents on endometrial edema patterns and serum estradiol 17-β levels has not previously been reported.

The objectives of the study were to use computer-assisted image analysis to characterize changes in the echotexture of the endometrium; to determine the best location within the uterus to analyse endometrial echotexture; to assess estradiol 17-β levels of the mare during the estrus; to describe how hormonal manipulation affected mean numeric pixel values (NPV) and mean estradiol 17-β levels; and to compare computer-assisted image analysis of endometrial echotexture of endometrial edema with estradiol 17-β levels.

2. Materials and Methods

Ten maiden, light horse mares that had a satisfactory breeding soundness evaluation, a negative uterine cytology, and a uterine biopsy grade of I or IIA, ranging in age from 2 to 6 yr and weighing between 400 and 500 kg, were used. The reproductive tracts were evaluated using an Ausonics Impact ultrasound scanner and a 6.0-MHz microconvex transducer every 12 h throughout estrus. The settings on the ultrasound machine that could influence the image attributes (i.e., power, overall time-gain compensation, near-field, and far-field gain) were standardized to predetermined values throughout the observational period. In an attempt to reduce the degree of signal attenuation, the distance between the transducer and uterus was minimized. Real-time and still-frame cross-sectional ultrasonic images of the uterine midpoints, tips, and body were recorded onto S-VHS tape with a Sears four-head VHS recorder (Sears Canada, Toronto, Ontario, Canada). Still-frame images were subsequently captured using a digital image acquisition system (PCVISION, Imaging Technology Inc., Woburn, MA) with a resolution of 640 × 480 pixels and 256 shades of grey. Captured images were analyzed using a SunSparc Station 10SX (Sun Microsystems, Mountain View, CA) and a computerized program designed specifically for the analysis of ultrasonic images (SYNERGYNE 10).

The echotexture of the endometrial layer of the uterus was evaluated using a spot metering measurement system. Artifacts such as areas of enhanced transmission and hyperechoic endometrial folds were avoided during spot placement. Each cross-sectional image of the uterus was divided into four quadrants. A spot measurement (25 mm²), encompassing approximately 20% of the total area of the endometrium in each respective quadrant, was evaluated. Spots were randomly placed in regions of the endometrium in each of the four quadrants (Fig. 1). The computer-generated output was expressed as two numeric values, mean NPV and SD (standard deviation). Mean NPVs (brightness of the pixel elements) were described by using a range of values from 0 (black) to 255 (white). The resulting mean NPV was the mean of the grey scale values of all the pixels falling within the measuring spot, and the SD was used as an indicator of image heterogeneity or the variability of the pixels within each individual spot measurement. An overall mean NPV for each cross-sectional image analyzed at each time interval was calculated using the average of the four individual mean NPVs obtained for each spot measurement. Hereafter, mean NPV refers to the average of the four spot measurements.

Estrus was defined as the period of sexual receptivity of the mare to a teaser stallion (daily teasing) and the presence of a 30-mm or larger follicle as detected by ultrasonography. Estrous behavior was rated on a scale of 0–4, with 0 corresponding to no sign of estrus and vigorous rejection of the stallion and 4 being a mare that postures, urinates, stands still, lifts her tail, and everts her clitoris. Blood samples were obtained at 6-h intervals throughout the entire estrus period until ovulation was confirmed by the presence of a corpus luteum. Serum estradiol 17-β was measured after solvent extraction in a radioimmunoassay, with a sensitiv-
ity of 1 pg/ml. The inter-assay coefficient of variation using spiked gelding serum at 25 pg/ml was 9.1% and at 50 pg/ml was 7.5%. Recoveries were in excess of 95%. The only other steroid metabolite in the assay that cross-reacted at greater than 1% was estrone at 10%.

Mares were subsequently examined during estrus over four successive estrous periods. Each successive estrous period was designed to examine the effects of a different degree of hormonal manipulation.

The four experiments were defined as follows:

Group 1: a natural estrous period with no hormonal manipulation
Group 2: a prostaglandin F2α (PGF2α)-induced estrous period dinoprost tromethamine, (5 mg SC). Prostaglandin F2α was administered regardless of follicular size on day 6 post-ovulation of the previous cycle
Group 3: a PGF2α-induced estrous period. Prostaglandin F2α was administered on day 6 post-ovulation and hCG, 2000 IU, IV, (APL) was administered to induce ovulation when the dominant follicle was ≥35 mm in diameter
Group 4: a PGF2α-induced estrous period. Prostaglandin F2α was administered on day 6 post-ovulation and hCG, 2000 IU, IV, was administered to induce ovulation when grade II endometrial edema was attained.

Group 1 served as an unmanipulated control group, and group 2 served as a PGF2α control group for the last two groups. Criteria for the use of the ovulatory induction agent in groups 3 and 4 were based on the desire to induce ovulation before spontaneous ovulation. Over the course of the study, 40 estrous periods were evaluated, consisting of four experimental groups with the same 10 mares in each phase of the experiment.

A statistical significance level of p < 0.05 was selected for all data analyses, and data points were adjusted by time before ovulation using day of ovulation as a reference point (day of ovulation = 0 h). Differences between mean NPV in locations within the uterus, SD of mean NPV (image heterogenicity), or serum estradiol 17β levels were evaluated over time from ovulation using a general linear, repeated measures analysis of variation (ANOVA). Mean NPV or estradiol 17β was the dependent variable, and time from ovulation was the independent variable. Post hoc comparisons between data points were made using the Bonferroni method in SPSS®. Differences in the length of estrus and peak estradiol 17β levels between groups were determined using Student’s t-test. The correlation (Pearson’s) between mean NPV and the corresponding estradiol 17β level was calculated for each group.

3. Results

Of the locations within the uterus, only the uterine body showed a change in endometrial echotexture over time from ovulation in groups 1, 3, and 4 (p < 0.001). There were no differences in image variability (p = 0.11, p = 0.09, p = 0.14, and p = 0.18 for groups 1–4, respectively). One mare (10%) in group 1, seven mares (70%) in group 2, two mares (20%) in group 3, and two mares (20%) in group 4 ovulated with minimal or no change in mean NPV. Mares that did not develop endometrial edema had lower estradiol 17β levels (p < 0.05) than mares that had changes in mean NPV. Figure 2 shows data from two mares; one mare did develop endometrial edema and had high estradiol 17β levels, and
the other mare did not develop endometrial edema and had low estradiol 17-β levels. Two mares (20%) in group 3 and three mares (30%) in group 4 did not ovulate within 48 h of hCG treatment and were identified as not responding to the hCG treatment. Mares that did not ovulate within 48 h of hCG treatment were followed until ovulation was confirmed. Data from these five estrous periods in which the mares did not ovulate within 48 h of hCG treatment were included in the analysis because there were no differences in the outcomes of interest for those that ovulated within 48 h of hCG administration and those that did not when the data were evaluated by time from ovulation. Figure 3 shows serial (every 12 h) ultrasound images of the endometrium of the uterine body in a single mare during estrus (A–K) and diestrus. Figure 4 is a graphical representation of mean NPV and mean estradiol 17-β levels over time before ovulation for each of the four groups.

Mares in groups 1 and 2 had a 6.0 ± 0.5 day estrus period, whereas mares in groups 3 and 4 had a 2.0 ± 0.5 day estrus period (p < 0.05). Estradiol 17-β levels were negatively correlated (r² = 0.63) with mean NPV. Peak estradiol 17-β levels were reached at −60, −144, −48, and −48 h in groups 1–4, respectively. The NPV of the uterine body, investigated by day from ovulation, changed (p < 0.001) over time in groups 1, 3, and 4. The pattern of change for mean NPV of the endometrium relative to the time before ovulation was influenced in groups 3 and 4 by hormonal treatment with hCG. Hormonal manipulation with hCG resulted in an accelerated rise and fall in mean NPV and an abbreviated endometrial edema curve. Estradiol 17-β levels changed over time as ovulation approached (p < 0.04) for all groups, and decreased throughout estrus in groups 2–4.

4. Discussion

Mean endometrial NPV changed significantly over time in only the uterine body. This suggested that the uterine body was the location within the uterus that should be used in clinical practice to assess changes in endometrial echotexture. It was our observation that the changes in endometrial echotexture were earlier in onset and more intense in the uterine body compared with the other locations within the uterus. In our study, mean echotextural values (NPV) of the endometrium in the uterine body (groups 1, 3, and 4) and mean estradiol 17-β levels for the 10 mares (groups 1–4) changed over time when the data were analyzed using ovulation as time 0. In the natural cycle, mean NPV values decreased from early estrus to a nadir at −96 h before ovulation and then increased to values similar to early estrus at ovulation. The similarity in mean NPV in early natural estrus and at ovulation suggested that the degree of endometrial edema was low at ovulation. Estradiol 17-β levels rose steadily throughout the natural estrus cycle, peaked 24 h before ovulation, and then decreased. Therefore, the decrease in endometrial mean NPV may be related to the decline in estradiol 17-β just before ovulation. The use of prostaglandin alone resulted in no significant change in endometrial mean NPV, and a steady decline in estradiol 17-β levels was noted throughout estrus. The levels of estradiol 17-β in group 2 at −144 h were similar to levels during natural estrus at −24 h. This may be caused by the variable length of estrus and follicular competence at the time of prostaglandin treatment or to the other effects of this compound. Hormonal treatment with hCG compressed the changes in endometrial echotexture into 48 h, and there was a steady decline in estradiol 17-β levels as ovulation approached. In groups 3 and 4, estradiol 17-β levels were initially low, which may have reflected the small size of the dominant follicle in these mares. In groups 3 and 4, estradiol 17-β declined until ovulation, which suggested that increased estradiol 17-β secretion was not needed for ovulation. Studies by Kerban et al. demonstrated that hCG induced changes in the theca interna of the dominant
Fig. 3. Serial (every 12 h) ultrasound images of the endometrium of the uterine body in a single mare during estrus (A–K) and diestrus (L: 144 h after ovulation). During early estrus, (A: 120 h; B: 108 h; C: 96 h before ovulation), at the time of ovulation (K) and diestrus, and 144 h after ovulation (L), the endometrial echotexture of the uterine body was ultrasonographically homogenous and devoid of overt folding. During mid-estrus (D: 84 h; E: 72 h; F: 60 h; G: 48 h; H: 36 h; I: 24 h; and J: 12 h before ovulation), the prominence of endometrial edema increased (D: 84 h; E: 72 h; and F: 60 h before ovulation). Maximal edema is represented by G (48 h before ovulation). This series illustrates a progressive increase followed by a decrease in the prominence of endometrial edema during a prostaglandin-induced estrus.
follicle. Kerban et al. suggests that the theca interna has reduced steroidogenic capability by 30 h after hCG.9 Our data support that the in vivo levels of estradiol follow the changes reported in in vitro studies of granulosa and theca cell molecular endocrinology. The fall in estradiol 17-β levels may be an indicator of the response to hCG. Prostaglandin treatment was administered to groups 2–4 and corresponded to the measurement of lower levels of estradiol 17-β. Further studies are required to determine if the lower levels of estradiol 17-β are caused by the effects of prostaglandin, the combination of the two hormones prostaglandin and hCG, or an error.

Generally, a roughly inverse relationship between the overall mean NPV and mean estradiol 17-β levels for the mares in each group was present: as mean estradiol 17-β levels increased, mean NPV decreased. We previously reported that endometrial mean NPV was inversely related to subjective edema score.2 In other words, when mean NPV and mean estradiol 17-β levels were plotted using the time of ovulation as 0 h, a peak in mean estradiol 17-β levels corresponded with a trough in mean NPV (Fig. 4). Therefore, a direct effect was observed between estradiol 17-β levels and subjective edema scores.

Endometrial echotexture, as assessed by mean NPV, was temporally associated with changes that have been historically reported in circulating estrogen levels. Changes in endometrial echotexture have been attributed to fluctuations in estrogen exposure, suggesting that a temporal relationship may exist between mean NPV and serum estradiol levels.1 Our data supported this hypothesis because we observed lower estradiol 17-β levels in mares with minimal changes in endometrial echotexture during estrus. Others suggest that endometrial edema occurs only in the presence of estradiol and in the absence of progesterone. Mares that failed to show changes in endometrial edema (echotexture) may have higher levels of progesterone, which causes partial or complete inhibition of the normal endometrial edema pattern.

Endometrial edema patterns were reported to be related to the number of estrogen receptors in the endometrium.10 During estrus, estrogen receptors in the endometrium increase, as diestrus approaches, estrogen receptors decrease.10 Therefore, a combination of shifting ovarian steroid ratios and a change in the number of estrogen receptors in the endometrium may be associated with resolution of
endometrial edema. The evaluation of this relationship between endometrial edema and levels of estrogen in individual mares lends support to this hypothesis.

We observed no statistically significant changes in the overall mean NPV over time in the prostaglandin induced estrus cycle (group 2). Administration of prostaglandin alone seemed to minimize the degree of echotexture changes observed in the endometrium. There was more variability in the time from prostaglandin treatment to response (onset of estrus) with a range of 1–7 days. The physiologic basis for this wide range in response times seemed to be related to the size and competence of the follicular structures in the ovary at the time of prostaglandin administration. Clinically, estrous behavior may be reduced or non-existent when a large follicle has ovulated within 24 h of administering prostaglandin. In addition, mares that ovulate within 24 h of prostaglandin injection are reported to have little or no subjective endometrial edema. The lack of change in mean NPV during estrus in group 2 may be caused by variability in follicular size and the lack of sufficient estrogen receptors in the endometrium at the time of drug administration, along with individual animal variability. Some mares in each group did ovulate with minimal change in mean endometrial NPV. No mares repeated this pattern; hence, the absence of endometrial edema was not inherent to individual mares. Rather, it was likely physiologically associated either with steroids produced from the dominant follicle or the systemic endocrine environment at the time of treatment.

Computer-assisted image analysis technology provides a non-invasive means of objectively evaluating the normal morphological changes in the endometrium of the mare during estrus. The use of mean NPV is presently limited by the limited availability of the software. Comparisons between mean NPV of the endometrium may be applied in clinical practice; however, it is important to standardize the ultrasound machine settings that influence image attributes.

In summary, in clinical practice the changes in endometrial echotexture should be evaluated in the uterine body. There is an inverse relationship between endometrial mean NPV and estradiol 17-β levels during estrus. While the majority of mares show a decrease and then an increase in NPV before ovulation, ovulation may occur with no change in NPV, and lower estradiol 17-β levels were observed in these mares. Hormonal therapy decreases estradiol 17-β levels, and hCG compresses the time frame for changes in mean NPV. This information may be useful for clinicians monitoring mares during natural or hormonally manipulated estrus. An integrated clinical management approach could be used to detect falling estradiol 17-β levels, and increasing mean NPV may be used in the future to help time insemination close to the time of ovulation.

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References and Footnotes


*Ausonics Impact, 6-MHz microconvex probe; Universal Ultrasound, Bedford Hills, NY 10507.
*Lutalyse, 5 mg, SC; UpJohn Pharmacia, Orangeville, Ontario L9W 3T3, Canada.
*APL, 2000 IU, IM; Wyeth Ayerst, St. Laurent, Quebec H4R 1J6, Canada.
*Synergyne 1, Dr. Roger Pierson, Royal University Hospital, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N 5B4
*Statistical Package for the Social Sciences (SPSS); Version 9.0; SPSS Inc., Chicago, IL 60606.