



Horse Care

by HEATHER SMITH THOMAS

Natalie Bragg-Wever, DVM, MSc (University of Saskatchewan) gave a presentation at the December 4-8, 2002 AAEP Convention in Orlando, Florida, discussing the changes that occur in the lining of the uterus when a mare comes into heat. Under natural conditions, many mares show some edema in the uterine lining as they come into heat, and the edema subsides as they get closer to ovulation. Many veterinarians have used these changes to help them predict when the mare might ovulate. But these changes can be altered and influenced by hormonal treatments given to mares (as when inducing them to come into heat more quickly), which may make it more difficult to accurately predict what is happening.

Edema in the endometrium begins at the onset of estrus, and these areas can usually be seen with ultrasound, says Bragg-Wever. Structural changes in the endometrium are associated with changes in ovarian creation of hormones, specifically estradiol (the most potent naturally occurring ovarian hormone). Computer image analysis can be used to objectively assess these structural changes in ovarian and uterine tissue.

"This assessment is based on the premise that various tissue densities reflect, transmit or propagate high frequency soundwaves. This allows us to evaluate ultrasound images with an enhanced precision of detail, because we can now assign one of 256 shades of gray to the area. This allows us to objectively evaluate changes in echotexture, rather than using the subjective method that we do in practice. This also allows us to scientifically quantify things, because it is objective, without an inherent bias," explains Bragg-Wever.

The objectives of her study were to utilize computer-assisted image analysis, to characterize changes in the echotexture of the endometrium, assess estradiol 17 beta levels of the mare during estrus, describe how hormonal manipulation affects the mean numeric pixel values and mean estradiol 17 beta concentrations, and compare mean numeric pixel values of the endometrium with mean estradiol 17 beta concentrations.

Ten reproductive tracts of young maiden light horse mares with healthy reproductive tracts (negative cytology and a uterine biopsy of either grade 1 or 2a) were evaluated over four successive

um were obtained, and the images were analyzed using a computerized image analysis program. Blood samples taken from the jugular vein were obtained every six hours throughout estrus, until ovulation. Serum estradiol beta was measured from these samples.

The echotexture of the endometrial layer of the uteri we reevaluated using a spot measuring technique. Cross sectional images of an ultrasound were divided into four quadrants for evaluation.

She showed graphs representing two mares, one with edema, and one without edema. In one mare that did develop

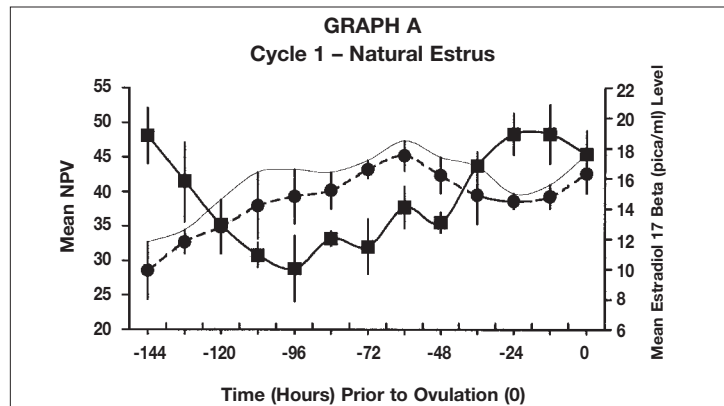
endometrial edema, the edema peaked at 36 hours prior to ovulation, and then the edema cleared as ovulation approached. "When you look at this mare's corresponding estradiol 17 beta level, we saw a gradual progressive increase as ovulation approached, with a peak at about 60 hours prior to ovulation, then a gradual decline," she says.

This was fairly typical in mares that devel-

oped edema in the uterine lining—a gradual buildup of edema, with maximum edema occurring about 36 hours prior to ovulation. The edema resolved (the fluid cleared out) much more quickly than it developed, being resolved by the time of ovulation.

In a mare that did not exhibit endometrial edema prior to ovulation, this was discernable from the ultrasound, but the estradiol 17 beta levels showed a gradual progressive decline as ovulation approached. "We looked at the ultrasound images every 12 hours. In a prostaglandin-induced cycle we have a longer duration of estrus."

"When we look at the natural cycle with no hormonal manipulation, there is roughly an inverse relationship—the estradiol levels increase as the edema decreases. In cycle two, which



Graphs A and B show the numeric pixel values (NPV) of the endometrium of the uterine body and the mean estradiol 17 beta levels for the 10 mares during two of the estrous cycles (ovulation = 0 hrs). The bold solid line represents the mean endometrial edema score determined ultrasonographically, while the bold dotted line represents the mean estradiol 17 beta levels.

cycles. The first cycle was one in which estrus occurred naturally and ovulation was spontaneous and not induced. The second cycle was prostaglandin-induced. Cycle three was again prostaglandin-induced using HCG (human chorionic gonadotropin) to stimulate ovulation, as was the fourth cycle. "We bought young maiden mares for this study, mares that had had no hormonal manipulations previously," says Bragg-Wever. Thus the first cycle would have no influence from estrus-inducing hormones.

Assessment was initiated at the onset of behavioral estrus, and when a dominant follicle reached a diameter of 30 millimeters. Mares were examined every 12 hours throughout estrus until ovulation was confirmed, and again on day six post-ovulation.

Still-frame images of the endometri-

was a prostaglandin-induced cycle, there was no development of endometrial edema in this group of mares. But in the mid-estrus period some mares developed pronounced edema patterns, while other mares did not develop any endometrial edema," she says.

The hormonal manipulation, particularly with HCG, shortened the duration of change. Generally an inverse relationship existed between the edema and the level of estradiol 17 beta concentrations. In other words, as the edema decreased, the systemic hormone level increased.

Mares were not always consistent, cycle to cycle, on whether they showed edema. "This is a little disconcerting, because sometimes in practice you tend to see mares that show repeatability—they traditionally don't show endometrial edema and so you identify them as a mare that doesn't show edema, and therefore may not evaluate them as closely as you should," she says.

"When we initially did this study we did think that in cycles three and four we were not going to see much endometrial edema patterns, based on practice exposure of looking at them at 48 hours and at 24, but we found that the endometrial edema is still present, occurring at a very abbreviated rate. So if you are not following them closely, you may not see it, and might classify that mare as not showing endometrial edema," she says.

"In this study we concluded that determination of endometrial echotexture and estradiol 17 beta during estrus should be evaluated when assessing young, reproductively normal mares for impending ovulation. Computer-assisted image analysis is a non-invasive method to objectively evaluate normal morphological changes in the endometrium. We suspect that convergence of computer technology, medical ultrasound imaging and endocrinology will aid in the

understanding of the ovulation process in the mare, which often still eludes us, as practitioners," she says.

If a person has a problem mare that is difficult to palpate or has a rectal tear or some other complication, the question was asked if there was an estrogen assay with quick turnaround time that would allow the veterinarian to monitor the mare for timing of HCG, for more proper timing of insemination.

Bragg-Wever answered by saying, "When you look at the overall estradiol level, you have to determine the critical turning point. For cycles three and four we had estradiol levels that started at a peak of 10 and went down to approximately eight. In cycle one

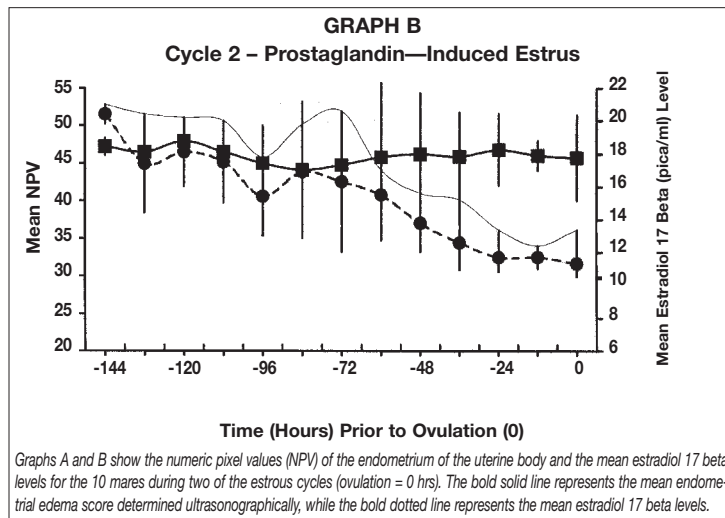
essentially interfered with our prediction ability," she says.

Ovuplant (a hormonal implant injected under the skin, on the neck) is preferred by some veterinarians over HCG, since repeated use of HCG does not work as well—many mares stop responding to the HCG. Mares given Ovuplant usually ovulate within 48 hours of the implant and can usually be bred the same day or the next day after its administration.

"With Ovuplant you'd anticipate fairly similar results, but I doubt you'd see endometrial edema. If we had the opportunity to do this study and look at a mare that had received HCG over three or four cycles, you may have a more direct comparison to the results with Ovuplant. This is why we designed the study the way we did, as a first exposure for these mares to hormone treatment, so there was no potential for antibody development to HCG. That may be why we saw more edema," she says. Mares that do not show endometrial edema in response to prostaglandin, in general, are usually mares that ovulate very quickly after prostaglandin injection.

In the Northern Hemisphere the mare has a short breeding season, so the study avoided spring and fall transition. "These four cycles were documented over a three-month period during peak season for breeding, to negate any potentially seasonal effect," she says.

"As practitioners in the field, I think a lot of us do rely, to some degree, on using endometrial edema to predict ovulation in the mare, but I think we have to reinvestigate that. When we are using it to predict ovulation, we must make sure we take into account what has been done to hormonally manipulate that cycle. This can really affect the type of changes we are looking for, as well as the degree of change," she says.



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they were a little bit higher, and went down to the critical point of eight. So the question is the interpretation, rather than the availability of the assay. I can't really tell you when that mare is going to go, but I am leaning toward a critical point of about eight," she says.

One part of the study looked at endometrial edema, and another part looked at follicular wall characteristics. "In the unmanipulated mare, we can definitely get within 12 hours for pinpointing ovulation. With the hormonal manipulation we can get within six to 12 hours, if they respond. That's the key. Right now we are looking at ways of identifying the mare that has received HCG that is not going to respond. These are the ones that