New Methods of Assessing Follicular Development and Predicting Ovulation in Mares

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Introduction

The prediction of impending ovulation by detection of changes in the reproductive tract is a challenge for veterinarians monitoring mares in both natural cover and assisted reproductive breeding programs. Careful breeding management of stallions and mares is required to maximize reproductive efficiency in programs involving: large books, limited breeding opportunities due to performance commitments, horses with physical disabilities, and assisted reproductive procedures such as artificial insemination (AI), embryo transfer, and hysteroscopic insemination. To maximize reproductive efficiency, mares are usually bred with a single insemination. The equine AI industry has been expanding as a result of the: development of passive cooling semen transport devices, an increase in rapid means of transportation such as courier service and airfreight, improvements in information technology, and breed association approval of cooled transported and frozen semen (Loomis 1999). For example the most populous horse breeds in North America, the American Quarter Horse and the American Paint Horse, approve the use of cooled transported and frozen semen. Continued growth and interest in AI is sustained by the large variety of breeds approving AI, cost effectiveness, and owner preference to transport semen rather than mares (Brinsko et al., 1999).

Serial evaluations of a mare=s reproductive tract are often required to determine readiness for breeding or impending ovulation. However practitioners report that it is challenging or difficult to service the needs of mare owners because of the intensive nature of serial reproductive examinations. Ultrasound examination of the mare=s reproductive tract is a relatively recent development that many veterinarians are just beginning to fully utilize. In addition, the regulation of equine estrus has historically been problematic because of the difficulties encountered in synchronizing estrus and ovulation, and in AI programs using cooled transported semen the challenge is to align mare ovulation with semen availability. Semen availability is influenced by the stallion=s collection schedule, courier or air freight delivery times, and clearance by customs officials. The use of frozen semen circumvents the problems with the availability of the semen, because it is stable in storage. Frozen semen is frequently marketed and sold by the insemination dose, rather than being sold as a breeding contract with a live foal guarantee. The longevity of frozen semen in the mare=s reproductive tract may be of shorter duration than that of cooled transported or fresh semen (Dobrinski et al., 1985). Pressure is placed on veterinarians to breed mares with frozen semen very close to ovulation with a single insemination because of the potential for decreased longevity of frozen semen and the practice of selling frozen semen by the insemination dose.

In summary in order to maximize reproductive efficiency and use only one insemination per cycle improved methods of regulating and predicting impending ovulation are needed. The detection of impending ovulation has many applications in a variety of breeding management programs. The following is a review of folliculogenesis in the mare, a description of the

traditional methods of assessing readiness to ovulate, and a discussion of new information on monitoring follicular maturation and detection of impending ovulation in mares.

A Review of Folliculogenesis in the Mare

Mares have both a luteal (diestrous) and follicular (estrous) phase of the estrous cycle. Each phase of the cycle is characterized by specific behavioral, physiological, endocrine, molecular and ultrasongraphic changes. The mean length of the equine estrous cycle is 22 $\pm$ 3 days, with the luteal phase 15 days for most mares, and estrous behavior expressed on 4.8 days (Hughes et al., 1975). Most of the variability in estrous cycle is due to changes in the length of the estrus. Pierson and Ginther (1985) and others reported that mares most frequently exhibited one wave of follicular development throughout the estrous cycle, while a small percentage of mares developed two follicular waves (Buratini et al., 1997; Ginther, 1983; Sirois et al.,1989). Follicular waves in the mare are divided into major and minor waves with further subclassification of the major wave into a primary and secondary wave (Ginther, 1983; Pierson and Ginther 1987). A major wave secondary arises in late estrus or early diestrus and results in a dominant follicle which either ovulates (diestrous ovulation) or regresses (dominant anovulatory follicle). The major primary wave arises during mid-diestrus in response to a surge in follicle stimulating hormone (FSH) and most often results in ovulation during estrus (Ginther, 1983; Bergfeldt and Ginther 1996). Follicles of the major wave grow in a synchronous manner until about mean (∀ SEM) of 7.4 ∀ 0.5 days prior to ovulation (Ginther, 1983), when the follicles diverge into a dominant and subordinate pool. The follicle that becomes dominant continues to grow and reaches a large diameter before ovulation (i.e., >40 mm). The subordinate follicles regress (Ginther, 1983). Ginther further reported that the dominant follicle of the secondary wave tended to have a smaller diameter than primary wave follicles (37.2 ∀ 1.0 versus 45.8 ∀ 1.0 mm) at the time of ovulation.

The Anatomy of the Dominant Follicle

The dominant follicle has an antrum, cumulus oocyte complex, and wall. The antrum is the interior of the follicle, which is full of follicular fluid. The follicle wall is composed of 2 main cell populations, the granulosa and theca cells. The granulosa cell layer lines the interior of the dominant follicle and cells from this layer comprise the cumulus oophorus which surrounds the oocyte. The next layer of the follicular wall is called the theca layer which has 2 sections divided by a basement membrane. The theca interna is well vascularized and is located beneath the granulosa cell layer, and a basement membrane separates it from the theca externa which is less vascularized.

Traditional Methods of Predicting Ovulation

Traditional methods of predicting ovulation include; the detection of softening of the dominant follicle by rectal palpation, serial measurements of the diameter of the dominant follicle, and monitoring changes in follicular characteristics such as: change in shape, wall thickness, wall echogenicity, wall separation, and presence of echogenic particles or following changes in endometrial edema (Ginther and Pierson 1984ab; McKinnon and Voss 1993; Griffin and Ginther 1991; Samper 1997).

Softening of the Dominant Follicle

Serial transrectal digital evaluation of the ovary is used to detect what has been described as: softening, a decrease in tensile strength, or a change in the consistency of the dominant follicle. Subjective softening of the follicle within 24 hours of ovulation was reported in approximately 70% of mares along with changes in follicular shape (Parker, 1971), but in contrast Hughes et al., in 1975 and 1977; reported softening in 28% of impending ovulations. Hughes *et al.* (1977) suggested that some follicles became turgid again just prior to ovulation, whereas others remained turgid throughout estrus and impending ovulation. Carnevale et al., 1988 reported that the preovulatory follicle had a decrease in follicular tone, which she hypothesized might be due to either a decrease in tensile strength of the follicular wall or a slow loss release of fluid from the follicle. Changes in follicular pressure of the dominant follicle have not been quantified**.**

Follicular Size

The most common means of assessing readiness to ovulate is serial measurements of the maximal diameter of the dominant follicle. Many report that the size of the dominant follicle is subject to seasonal, breed, and individual mare variability. Mean follicular sizes at ovulation early in the breeding season are larger than later in the breeding season. Draft mares are reported to have larger follicular sizes at ovulation (55 mm) (Plata-Madrid et al.., 1994).. Follicular size at ovulation is quite variable and may range from 35 B 55 mm in light horse mares, with a mean follicle size of close to 45 mm (Pierson and Ginther 1985; Pierson and Ginther 1987; Bergfeldt and Ginther 1996). However, mares may ovulate follicles smaller or larger than the aforementioned sizes, and follicular size at ovulation may vary in an individual mare (Newcombe 1996). The dominant follicle typically grows at a rate of about 2 - 3 mm per day, however just prior to ovulation follicular size may increase, decrease or remain static. Carnevale et al., 1988 reported that the diameter of the dominant follicle decreased 13% from 30 minutes prior to ovulation until the beginning of ultrasonographically detectable ovulation. Gastal et al., 1998a,b reported that changes in the echotexture characteristics of the follicular wall were more reliable than a measurement of follicular size in identifying the optimal day of breeding in mares.

Follicular Characteristics

Changes in the characteristics of the dominant follicle visible by ultrasonographic examination of the mare include: an irregular follicular shape, a thickening of the follicular wall, increased echogenicity of the follicular wall, the presence of an anechogenic layer in the follicular wall, particulate matter in the follicular antrum, and a tear in the follicular wall (McKinnon and Voss, 1993). The percentage change in follicular shape, size of the follicle, and thickness of the follicular wall, have been evaluated as a means to predict ovulation (Pierson and Ginther, 1985). Carnevale et al., 1988 reported that the preovulatory follicle tended to flatten which resulted in the production of irregular images on ultrasonography. Others report that the flattening observed in the preovulatory follicle may result from additional pressure put on the ovary to achieve good contact with the ultrasound probe due to decreased follicular tone (Newcombe 1996). Follicles are reported to loose their spherical shape and become more triangular or coned toward the region of the ovulation fossa. Pierson and Ginther (1985) described a thickening of the follicular wall prior to ovulation. Gastal et al., 1998a describes the

presence of an anechoic line in the follicular wall. He reported that the anechoic line indicates separation between the granulosa and theca layers of the follicle, and that the length of the anechoic line increases as ovulation approaches. Additionally, an increase in the echogenicity of the follicular antral fluid and follicular wall has been observed prior to ovulation (McKinnon and Voss, 1993; Gastal et al., 1998b).

Changes in Endometrial Edema

Follicular estradiol secretion was hypothesized to have a direct relationship with the changes in endometrial edema (Ginther 1979; Hayes et al., 1985). An evaluation of the degree of endometrial edema has been suggested to indicate follicular responsiveness to hCG (Samper 1997). The degree of endometrial edema was reported during estrus in mares by many authors including Pierson and Ginther 1984; Hayes et al., 1985; and Griffin and Ginther 1991). The changes were described by using a 5 point scale. Each increment on the subjective scale is given a grade with a range of 0 to 4. Grade 0 represents no edema, while grades I to IV represent increasing amounts of endometrial edema. In early estrus edema is light (grade I) and then increases in mid-estrus to grade II. In an unmanipulated estrus maximal edema (grade III or grade IV) is reached in late midestrous at about 72 to 96 hours before ovulation. Endometrial edema then decreases from maximal levels until about 36 hours before ovulation when edema is light or absent. Figure 1 has two ultrasound images of the cross section of a uterine horn, with a diestrous endometrium with a grade 0 edema, and a late midestrous endometrium with grade IV edema.

A: Diestrus **B: Estrus**

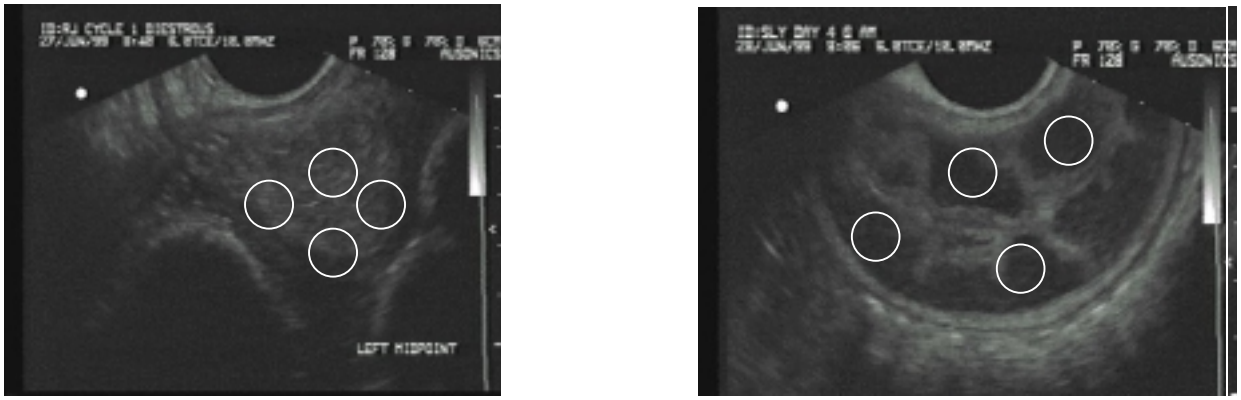


Figure 1: An example of the echotexture of the diestrous (A) and estrous (B) equine endometrium with spots placed in a cross-sectional image of a uterine horn. The echotexture of A is homogenous while B shows prominent anechoic regions characteristic of grade IV (maximal) endometrial edema. A circle of a predetermined area (i.e., 25 mm^2), encompassing approximately 20% of the total area of the endometrium in each respective quadrant of the uterus, was used to evaluate echotexture of the equine endometrium.

Other authors refer to the changes in endometrial edema as changes in endometrial folding patterns. The interior of the uterus has folds of endometrial tissue. The periphery of the

folds increase in size as the amount of edema increases, rather than the folds going from flat to folded or folding up per se.

Effect of Hormonal Manipulation

The impact of hormonal manipulation such as the use of prostaglandin to advance estrus, and hCG and deslorelin to induce ovulation on follicular or endometrial characteristics has not been completely described. The objective behind the use of hCG in mares or a GnRH analogue (deslorelin, leuprolide) is to reduce the number of matings/inseminations per estrous cycle and to increase the likelihood of ovulation occurring close to insemination (Bott and Bailey, 1996). Human chorionic gonadotrophin administration in early estrus shortens the duration of estrus, so that in those animals which respond, ovulation occurs within 48 hours of treatment, with a range of 24 to 48 hours, with the mean time to ovulation as 36 hrs. It has been reported that up to 85% of the mares ovulate by 48 hours after treatment with hCG (Duchamp et al., 1987) (Sullivan et al., 1973; Loy et al., 1966). Gastal et al., 1998b reported an increase in follicular wall echogenicity in mares treated with hCG. A successful response to hCG administration has been shown to be associated with strong standing estrous behavior, a minimal follicular size of 30 mm at the time of administration and the presence of well developed endometrial edema (grade II to III) (Bollwein and Braun, 1999).

When prostaglandin is used to induce estrus, the variability in the treatment to estrus interval is dependent, in part, on the status or presence of the follicles on the ovary at the time of injection. Possible outcomes in mares treated with prostaglandin when they have a large follicle include: ovulation in 24 hrs with minimal or no signs of behavioral estrus, the mare has an estrus of normal length but ovulates a very large follicle, or the mare comes into heat quickly and has a short estrus (Douglas and Ginther 1972; Loy et al., 1979).

New Methods of Detecting Follicular Maturation

Diagnostic ultrasonography enables a practitioner to objectively assess uterine and follicular changes. However, with the advent of computer-assisted image analysis, routine diagnostic ultrasonography is becoming revolutionized because interpretation of an image is no longer limited by one=s ability to detect differences in the grey scale image. With the aid of a computer, tissues or areas of interest in an ultrasonographic image can be quantified with enhanced precision of detail, through the evaluation of grey-scale pixel differences (Pierson and Adams, 1995). This technique has been applied to analyze ovarian tissue of women, mares and cows (Hanna 1999; Tom et al., 1998; Bragg et al., 2001). Convergence of digital and wireless technology has influenced the way medical information is now generated, stored and processed. Many ultrasound machines are now available that produce an image in a digital format. The coupling of computer-assisted image analysis with ultrasound image analysis is a logical development in the application of diagnostic ultrasound.

Each ultrasound image is composed of an array of pixels of varying shades of grey. The human eye can perceive only 8 to 10 shades of grey. Routine ultrasonography produces images with 64 shades of grey, while a higher quality ultrasonogram or with the use of the computer assisted image analysis system as an ancillary tool, each pixel in the image can reflect one of a possible 256 shades of grey (Pierson and Adams, 1995). With computer-assisted image analysis, routine diagnostic

ultrasonographic images are converted to a digitized format (Gauthier *et al.*, 1992). Digitization is a process whereby each pixel of an image is assigned a numeric value for brightness. Digitization can involve the use of a high resolution acquisition board and a narrow band width amplifier (Pierson and Adams, 1995). Acquisition boards can be used to increase the brightness and contrast resolution of the images. These changes are frequently associated with concurrent alterations to detail, edge enhancement and texture (Pierson and Adams, 1995), a process that is often collectively referred to as image acquisition. Acquisition often results in improvements to image quality, thereby, allowing subtle changes to be visualized. Each portion of a digitized image can then be evaluated to allow quantification of pixel intensities (grey-scale) and/or the distribution of pixel intensities (standard deviation or a measure of pixel heterogeneity) (Pierson and Adams, 1995). Pixel information is analyzed to reveal information about the physiologic function of the tissues.

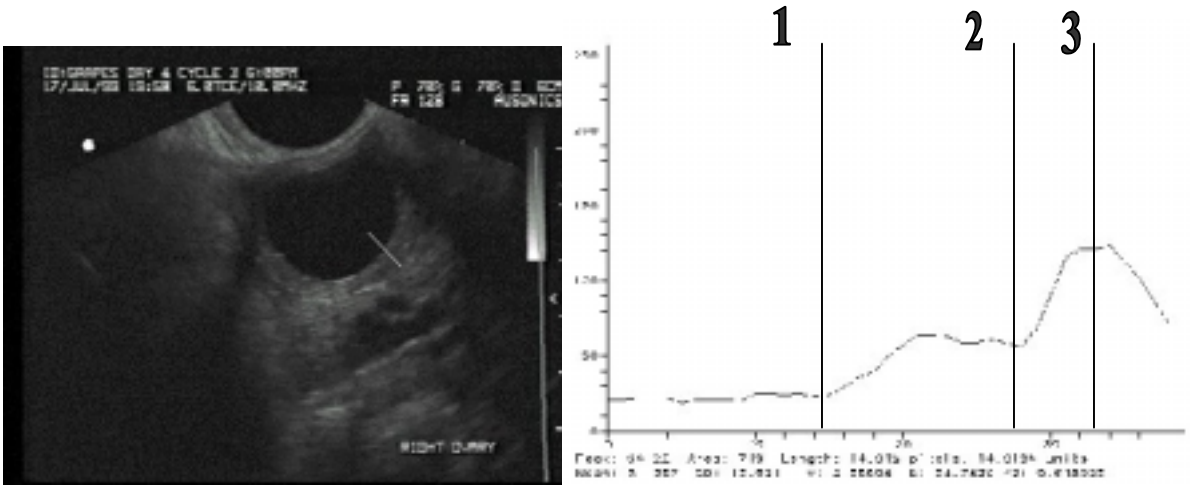
One of the easiest methods of assessing pixel information is the use of sample spots of a predetermined size to evaluate the tissue of interest. Spot measuring is one of the methods used to quantify pixel values in a particular area of interest. Spot measuring uses a circle with a predetermined diameter to measure echotexture, in terms of mean numeric pixel values of the pixels in the spots, in a user-selected area of interest such as the endometrium or follicular wall (Figure 1). A computer is used to precisely determine the numerical value that corresponds to the grey-scale of each pixel within the spot (Pierson and Adams, 1995). The output is expressed in terms of a mean numeric pixel value or NPV, plus or minus a corresponding standard deviation (Pierson and Adams, 1995). An average can then be calculated by obtaining a multiple number of randomly predetermined spot measurements. This allows mean pixel intensities of images to be compared at various points in time. For example, in the mare, one could compare mean NPV of the endometrium or follicular wall for different time points in the estrous cycle. These comparisons would enable investigation of the changes in the pattern of endometrial edema throughout estrus.

A user-selected section of the follicular wall can be evaluated with the use of a computer-assisted image analysis program employing a line drawn through a user-selected section of the ovary, thereby, allowing pixel values to be quantified along the line. This process is referred to as a linear analysis (Pierson and Adams, 1995). A graph is then generated that is a plot of pixel intensity by pixel length. The amplitude of the echoes along the line drawn through the point of interest are plotted, and in the case of the ovary the line usually in drawn through the antrum, follicular wall and ovarian parenchyma (Pierson and Adams, 1995). Figure 2 shows an example of a linear analysis of a dominant follicle. Because the linear analysis evaluates the pixels that fall on the line, an additional improvement would be the evaluation of a small area of tissue to generate mean values of a larger number of pixels. The grey scale may be replaced with color scale to help enhance specific features of the follicle. Figure 3 shows an image of a follicle and a three dimensional contour image of the same follicle. In figure 3 a color enhancement more readily allows the detection of the anechoic line in the follicular wall is described by Gastal et al., 1998b. The computer is used to create a grid where length, width, and height of the pixel intensities in the selected region, which in this case is the follicular wall, are plotted to provide the three dimensional image.

Newer ultrasound machines produce better sharper images that allow more detail to be evaluated. The presence of an anechoic line in the follicular wall is an example of a characteristic not previously evaluated. In mares treated with prostaglandin, the presence and extent of the anechoic line in the follicular wall was reported by Gastal et al., 1998b in relationship to ovulation. They also , report that there is seasonal variability in the detection of the anechoic line. It may be

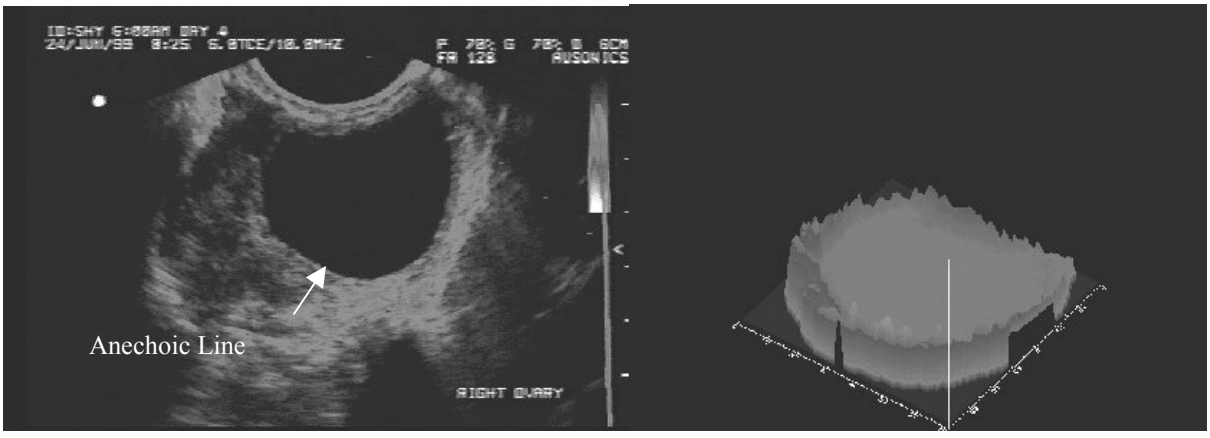
visible up to 3 days before ovulation. Our observation is that it requires practice to correctly identify this structure. Physiologically we feel the anechoic line may be related to the collapse of the thecal interna layer. Gastal et al., 1998 also reports on the detection of particles in the follicular fluid, which he suggests represent granulosa cells close to ovulation.

Endocrine changes, in hormones such as estradiol 17 β, have been discussed as a means to indicate follicular maturity (Allen et al., 1995), but daily blood samples would be required.



A B

Figure 2: Linear analysis of a dominant follicle. The pixel intensities of the white line in A have been plotted in B. The pixel intensities of the pixels falling on the line are plotted from the follicular antrum towards the ovarian parenchyma. The X axis in B is pixel length, and the Y axis is pixel intensity. The area under number 1, 2 and 3 in B corresponds to the follicular antrum, wall and ovarian parenchyma, respectively.



A B

Figure 3: Enhanced image of an ultrasonographic image (A) and three-dimensional contour image of same follicle (B). The anechoic line is identified by the arrow in A. The three dimensional contour image illustrates the differences in the thickness of the follicular wall at various points.

Treatment with hCG decreased peak levels of estradiol. Peak estradiol levels are reached before ovulation and levels are highest in spontaneous estrus.

Follicular Tonometry

Follicular tonometry is a large scale adaptation of the technique and equipment used during ophthalmic examinations to determine intraocular pressure. Factors that were proposed to influence follicular pressure include: size of the dominant follicle, the position of the follicle on the ovary, arrangement of surrounding subordinate follicles, the percentage of ovarian tissue invested by the ovulation fossa, and follicular maturation (Carnevale et al 1988). We were able to detect a small decrease in pressure in the -24 to -12 hour time period before spontaneous and hCG induced ovulations (Bragg et al., 2001). Follicle pressure was variable before the preovulatory decrease, hence serial evaluations were necessary to detect the decrease in pressure. The magnitude of the decrease in follicular pressure was 0.5 - 1.0 PSI which is relatively small change. We did not believe that the drop in pressure was sufficient to explain the change in follicle tone that individuals report prior to ovulation by manual palpation. The perception of softening may relate to other physiologic events such as changes in the follicular wall including a decrease in tensile strength, decrease in follicular fluid density, or hemorrhage and edema in the theca interna. The relationship between follicular maturity and pressure requires further investigation. A method to detect wall deflection will help determine if there is a change in tensile strength in the follicular wall.

Effect of Hormonal Manipulation on Assessing the Interval to Ovulation

We have used transrectal tonometry and computer assisted ultrasound image analysis to characterize changes in 10 mares during natural estrus(cycle 1), prostaglandin induced estrus (cycle 2), and prostaglandin induced estrus combined with hCG treatment to induce ovulation when endometrial edema was grade II (cycle 3) or when a follicle of >35 mm in diameter was detected (cycle 4). In our studies we evaluated follicular size because of its common use as an indicator of follicular maturation. In unmanipulated estrus (n = 10) the dominant follicle grew at a rate of 2.5 $\pm$ 0.6 mm/day. The mean periovulatory diameter of the dominant follicle was 45.2 $\pm$ 1.7 mm. Follicular growth rates during the last 24 hours prior to ovulation were suppressed as the size of the dominant follicle: remained static, grew at a decreased rate, or slightly decreased prior to ovulation. The echotexture of the follicular wall obtained by spot measurements to generate a mean follicular wall NPV, was variable during estrus. The follicular wall at the base, the point opposite the place of ovulation (the ovulation fossa), exhibited a progressive increase in thickness as ovulation neared in the unmanipulated estrous group, however this change was small. Follicular pressure of the dominant follicle, assessed at the base of the wall, fluctuated through the days prior to ovulation. However, a significant decrease in follicular pressure was noted between the 24 and 12 hr intervals prior to ovulation.

The uterine body was the best location within the uterus to assess endometrial edema. However in our study there were some mares in each cycle that did not develop edema. Mean endometrial NPV reached a maximal levels at 96 hours before ovulation (low NPV), then decreased until 36 hours (high NPV) before when the mean NPV was the same as in diestrus suggesting no edema was present.

Mares which were induced to come into estrus through the administration of a luteolytic agent, Lutalyse, 5 mg SQ $PGF_{2,}$ did not exhibit a significant change in endometrial

NPV over time prior to ovulation. The majority of mares (7/10) that were given prostaglandin on day 6 post ovulation did not exhibit any change in endometrial edema (NPV) throughout estrus. The three mares which did exhibit edema in this group exhibited patterns which were similar to the mares which did not receive a luteolytic agent. The mares with minimal change in mean endometrial NPV had lower levels of estradiol (Bragg Wever et al., 2002). We believe that the lower level of estradiol resulted in less edema in the tissue and correspondingly less of a change in endometrial echotexture. We observed that the peak levels of estradiol were lower in mares treated with prostaglandin than in mares during unmanipulated estrus (Bragg Wever et al. 2002). The mean rate of growth of the dominant follicle was 1.9 $\pm$ 0.5 mm/day until a mean periovulatory diameter of the dominant follicle was 46.9 $\pm$ 4.3 mm. Similar to the mares which were not manipulated during estrus, follicular growth rates during the last 24 hours were suppressed in the prostaglandin treated mares. The time frame from injection of prostaglandin to ovulation was variable and was related to the size of the follicle on the ovary at the time of prostaglandin administration. The follicular wall echotexture gradually increased over time prior to ovulation. The average daily rate of change was 3.1 $\pm$ 1.9 pixels. Changes in mean NPV less than 10 pixels are subtle and challenging to detect. The underlying cause of this increase in follicular wall NPV remains speculative. It is not known if this is a direct, indirect or chance observation. The follicular wall is not homogenous because of the 2 layers composed of granulosa and theca cells. The treatment with prostaglandin was on day of diestrous, hence there were a number of days before ovulation. The hormone PGF_2 is reported to have a short half - life. It seems unlikely therefore that the increase in follicular wall NPV is directly related to prostaglandin treatment. The follicular wall at the base exhibited a progressive increase in thickness as measured by the area under the curve, as ovulation neared in the PGF_2-induced estrous group with a mean change of 71.3 $\pm$ 24.6. This is a relatively small change in wall thickness that is not necessarily discernible with the naked eye. As per the unmanipulated estrous group, follicular pressure of the follicle wall fluctuated through the days prior to ovulation. Follicular pressures of the dominant follicle decreased in a similar manner as to the mares in the unmanipulated estrous group. This change occured despite lower levels of estradiol suggesting that high peak levels of estradiol are not necessary for the drop in follicular pressure. In addition mares treated with prostaglandin were less likely to develop endometrial edema.

Mares which were induced to come into estrus with the administration of PGF_2 on day 6 post ovulation and then treated with hCG to induce ovulation had a shortened duration of estrus. The mean duration of estrus was 2.0 $\pm$ 0.5 days compared to 6.0 $\pm$ 0.5 days in the two groups which did not receive hCG. Human chorionic gonadotrophin was administered based on follicular size in cycle group3. Changes in endometrial edema, in terms of mean endometrial NPV, for mares in this group were abbreviated when compared to the unmanipulated group. In cycle group 3 mares mean endometrial NPV were lowest at 36 hrs prior to ovulation, while endometrial edema had resolved by 12 hrs prior to ovulation, these same time points were reached in the unmanipulated cycle at 96 hrs and 36 hrs respectively.

The dominant follicle had a mean rate of growth of 1.7 $\pm$ 0.1 mm/day until a mean periovulatory diameter of 39.2 $\pm$ 1.3 mm was achieved. Follicular growth rates during the last 24 hours were suppressed similar to the other groups. Follicular wall echotexture in group 3 mares gradually increased over time prior to ovulation with a mean daily rate of change of 11.7 $\pm$ 5.3 pixels (Bragg et al., 2001). The physiologic changes that result in this increase in mean follicular wall NPV have not been identified. Prior to ovulation there are changes in the granulosa cell

layer suggesting luteinization and some authors report an increase in the production of a mucus secretion. Changes in follicular wall tissue elements, and tissue interfaces, may result in an increase in echogenicity. Concurrent histologic studies of follicles and their ultrasound attributes may help elucidate the basis for these changes. The base of the follicular wall did not change in thickness over time prior to ovulation, although the general trend for the wall at this point was to increase as ovulation neared. Similar to the other groups, follicular pressures fluctuated through the days prior to ovulation and decreased between the 24 to 12 hr interval prior to ovulation.

Mares in the last group were treated with PGF_2 to hasten estrus, and were administered hCG to induce ovulation. The administration of hCG was based on the attainment of grade II endometrial edema. Changes in mean endometrial NPV for this group occured in a shorter span of time, i.e. were abbreviated, when compared to the unmanipulated estrous group and resembled the pattern of endometrial edema in the group 3 mares (hCG was administered based on the dominant follicle attaining a diameter of 35 mm). The peak of endometrial edema occurred slightly later in group 4 (24 hrs) when compared with group 3 (36 hrs), although a return of mean endometrial NPV to the values obtained in diestrus was obtained at the same time prior to ovulation in groups 3 and 4 (12 hrs). The dominant follicle grew at 4.0 ± 0.1 mm/day until a mean periovulatory diameter of 43.0 ± 2.5 mm was reached. Follicular growth rates during the last 24 hrs were suppressed as in the other groups. The echotexture of the follicular wall progressively increased and appeared brighter over the time prior to ovulation with an average daily change of 10.7 ± 4.3 pixels. The increase in mean follicular wall NPV was present in cycle groups 2 - 4. The hormonal treatment common to all cycle groups is prostaglandin. It is possible that the combination of prostaglandin and hCG may have the same effect on the follicular wall echotexture as prostaglandin alone, but hCG does not appear to enhance this process in follicles that are small at the time of treatment. The follicular wall at the base did not thicken, although similar to cycle group 3, there was a trend toward increasing values. Follicular pressures fluctuated through the days prior to ovulation, with a decrease within the last 24 hrs prior to ovulation. While we did not do statistical comparisons between groups, with the data aligned by day from ovulation we observed that the pressure measurements of the dominant follicle were higher in the days prior to ovulation than for the mares in the group in which hCG was administered based on the diameter of the dominant follicle. The higher pressures were associated with follicles with a smaller diameter, which on palpation were firmer.

Summary:

A combination of follicular and uterine attributes should be assessed to determine the optimum time for breeding, and consideration should be given to the impact of any hormonal manipulation the mare received (see points below). The follicular and uterine attributes that should be assessed may include: follicle size, presence of an anechoic line in the follicular wall, an increase in follicular wall echogenicity and a decrease in follicular tone. Computer assisted image analysis and follicular tonometry are still in the developmental phase but these emerging technologies may be available in the future to objectively monitor changes in the dominant follicle and endometrium of the uterus.

Follicular and Uterine Attributes

1. Measurement of maximal antral diameter is recommended because it is easy to quantify and has a good correlation with follicular maturation. In the 12 hours before ovulation

the dominant follicle may increase, decrease, or stay the same size. The use of hCG to induce ovulation when the follicle size was small resulted in the ovulation of follicles with a large size, suggesting they may grow at an increased rate.

2. The use of prostaglandin on day 6 post ovulation is associated with lower circulating levels of estradiol and minimal changes in endometrial edema in the induced estrus. In a prostaglandin induced estrus changes in endometrial echotexture, and endometrial edema are less obvious or absent when compared with spontaneous estrus. A small percentage of mares treated with or without hormonal agents may show minimal changes in endometrial edema before ovulation.

3. Follicular wall thickening is more prominent in the base of the dominant follicle in natural estrus and PG induced estrus. The anechoic line is more prominent in the basal region of the dominant follicle.

4. Mares treated with prostaglandin, or hCG had more echogenic (brighter) follicular walls than mares in natural estrus.

5. Computer assisted ultrasound image analysis of endometrial echotexture NPV are inversely parallel to the reported changes in subjective endometrial edema grades.

6. Endometrial edema score is best determined by evaluating the body of the uterus. A decrease in endometrial edema usually occurs before ovulation, however the time frame for the decrease is influenced by hormonal treatment. The use of hCG in early estrus accelerates the changes in endometrial edema.

7. Tonometric detection of follicular pressure indicates that there is decrease in follicular pressure -24 to -12 hours before ovulation The decrease in pressure occurs during: natural estrus, prostaglandin treated, and prostaglandin and hCG treated mares.

8. Future studies are required to determine the usefulness of computer assisted ultrasound image analysis. Concurrent evaluations of ovarian histology, and ultrasonographic features of the ovary are required to further determine the structural basis of follicular wall changes.

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