Clinical relevance of follicular dynamics for optimal reproductive management of cycling mares

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Abstract

Reproductive hormones, such as prostaglandin F_2 alpha, are widely utilized in equine reproductive management. The use of transrectal palpation and ultrasonography, along with an understanding of the physiology of follicular dynamics, and appropriate use of those reproductive hormones, help the equine practitioner better manage single mares for breeding or even groups of mares requiring estrus and ovulation synchronization for breeding or advanced reproductive techniques. Much research has illuminated critical events that occur during the diestrus period in the mare that assist the practitioner in making the most appropriate treatment decisions. This paper reviews the information gained from that research allowing practitioners to incorporate the knowledge into everyday reproductive practice.

Keywords: Follicular dynamics, prostaglandin F2alpha, cycling mare, estrus synchronization, ovulation synchronization

Introduction

With the discovery that prostaglandin F_2 alpha ($PGF_{2\alpha}$) is the endogenous luteolytic hormone in sheep,[1] and later in the horse in the early 1970's,[2,3] a powerful new dimension of reproductive management was added to the toolbox of the equine practitioner (i.e. the ability to "short-cycle" a mare by abbreviating the luteal phase). However, this discovery was before ultrasonographic examination of the reproductive tract of the mare was possible and the physiology of follicular dynamics (i.e. primary and secondary follicular waves) were fully understood, which contributed to the occurrence of frustrating inconsistencies in mares following treatment with $PGF_{2\alpha}$. The use of transrectal palpation and ultrasonographic examinations of the reproductive tract of the mare has partially eliminated these apparent frustrating inconsistencies by allowing the practitioner to better know the ovarian/follicular status of the mare. For example, the mare that is presented with a history of showing recent estrous behavior and ultrasonographic evidence of a corpus luteum (CL) without a secondary dominant follicle is generally not challenging to the practitioner. In contrast, the mare that is presented with a history of exhibiting recent estrous behavior, has ultrasonographic evidence of a CL, and also has a large (>30 mm) secondary dominant follicle (i.e. a "diestrus follicle") can be challenging to manage. Therefore, this review will focus on how to optimally manage the cycling diestrus mare, based on the current understanding of follicular dynamics and its impact on how an individual mare responds to exogenous $PGF_{2\alpha}$.

Physiology of primary and secondary follicular waves

Mares are considered monovular, since they predominantly develop one ovulatory follicle during estrus.[4] Terminology referring to the events associated with follicular dynamics throughout the estrous cycle are listed in the Table.[5] All cycling mares have a primary major follicular wave that begins in late diestrus resulting in the selection of the dominant (ovulatory) follicle. That follicle is associated with the return to estrus after luteolysis causes regression of the CL that originated during the previous ovulatory estrus (Figure1[6]). It is important to remember the progression of an ovarian follicle within a follicular wave during the ovulatory season as depicted in Figure 2.[6] Some mares only develop the ovulatory primary follicular wave (top panel Figure 1) as can be seen ultrasonographically with a single dominant follicle and edema of the endometrial folds where the dominant follicle will most often proceed to ovulation (Figure 3).[7] Other mares will develop an earlier (in diestrus) major secondary follicular wave that is associated with selection of a dominant (≥25mm) follicle in mid-diestrus that, without

pharmacological intervention, will have one of three outcomes: 1) regression (atresia), 2) "silent" ovulation (incidence ranges from rarely in ponies,[8] 4% of cycles in Quarter horses and Appaloosas,[9] to 25% of cycles in Thoroughbreds[10] and Standardbreds[11]), or 3) development into a hemorrhagic anovulatory follicle (HAF)[7] (incidence range from 2.5%[12] - 35.8%[13]) as shown in the middle panel of Figure 1. These mares have a functional CL and a secondary dominant follicle, but are not physiologically or behaviorally in estrus due to progesterone being the dominant hormone when both progesterone and estrogen are being secreted (Figure 4).[14,15] A third group of mares will develop a secondary minor wave that is not associated with selection of a dominant follicle (the largest follicle remains <28mm), as shown in the bottom panel of Figure 1. The varying degrees of follicular development among each "type" of mare dramatically influences their response to a luteolytic dose of $PGF_{2\alpha}$ (i.e., how soon they come into behavioral estrus and ovulate). Therefore, it is essential to know the follicular status of an individual mare at the time of $PGF_{2\alpha}$ administration for optimal reproductive management.

The importance of knowing the ovarian follicular status of mares when administering $PGF_{2\alpha}$

Given the variability of follicular wave patterns among mares, it is important to know the status of the ovaries in mares at the time of $PGF_{2\alpha}$ treatment. This will enable the practitioner to more accurately predict the mare's response following administration of $PGF_{2\alpha}$ (i.e. approximately how soon she will come into estrus and ovulate).[12,16]

The challenge is to predict (to the degree possible) the expected interval from $PGF_{2\alpha}$ treatment to ovulation. When treating with $PGF_{2\alpha}$, the interval from treatment to ovulation is related to the mare's follicular wave status (i.e. size of ovarian follicles at the time of treatment as shown in Fig. 1).[17-19] Burden et al. recently confirmed the findings of early studies, showing an inverse relationship between the size of follicle and the interval of treatment to ovulation (i.e. the larger the size of the diestrus follicle at the time of $PGF_{2\alpha}$ treatment, the shorter the interval to estrus/ovulation).[12]

As noted above, the potential outcomes of large secondary diestrus follicles are regression, ovulation, or formation of HAF. Therefore, treating a mare with $PGF_{2\alpha}$ without knowledge of the ovarian status could result in unexpected outcomes based on the presence of a large secondary diestrus follicle.

The potential outcomes of $PGF_{2\alpha}$ treatment in a diestrus mare

Knowledge of the ovarian status of mares under consideration for $PGF_{2\alpha}$ treatment allows the practitioner to make informed management decisions. Research has shown that some variation exists as to when the luteal cells of the equine CL will be responsive to single dose of $PGF_{2\alpha}$.[2,3,20,21] It is currently common practice to treat recently ovulated mares no sooner than day five of the cycle (ovulation equals day 0).[17,19,22] This point supports the importance of using transrectal palpation and ultrasonography to determine the ovarian status of the mare. Given the wide variation in length of estrus in mares (three to seven days, or longer) and the fact that signs of estrus continue for 24 to 48 hours after ovulation,[23] it becomes difficult to precisely determine when the CL is "mature" and sensitive to a single dose of $PGF_{2\alpha}$ without knowing the day of ovulation.

When emergence of a secondary follicular wave does not occur in early diestrus, there will not be a large secondary dominant follicle present, so mares will have minimal follicular activity at the time of $PGF_{2\alpha}$ administration (Figure 5). Once the CL is mature, five days after ovulation, treatment with one single administration of $PGF_{2\alpha}$ should reliably result in luteolysis, causing the mare return to estrus in three to five days.[2] Based on the experience of the authors, the interval from treatment to ovulation in mares with this follicular status can be longer than ten days, which is consistent with data from Burden et al., which showed that when all secondary follicles were <10mm in diameter, the average interval to ovulation after treatment with $PGF_{2\alpha}$ was 11.8 days.[12] This situation occurs when emergence of the next primary follicular wave has not yet occurred, meaning no follicles >10 mm are present at the time of treatment (Figure 5).

In contrast, mares that have a secondary follicular wave in early diestrus and develop a large secondary dominant follicle will generally have a shorter interval from $PGF_{2\alpha}$ treatment to ovulation.

Burden et al. determined that the interval to ovulation after treatment with $PGF_{2\alpha}$ for secondary follicles <35 mm in diameter was inversely proportional to the diameter of the largest follicle present at the time of treatment.[12] Secondary follicles 20-24 mm and 30-34 mm in diameter had an average interval to ovulation after treatment of 9.1 days and 7.8 days, respectively.[12] Therefore, a mare with a 34 mm follicle on the left ovary, a CL on the right ovary, and lack of edema of the endometrial folds (Figure 6) would have an expected interval from treatment to ovulation of approximately 7.8 days. Secondary follicles that are large (≥35 mm) can have a treatment to ovulation interval of 48 hours.[12] Based on the research, a mare with a ≥35 mm follicle on the right ovary, smaller follicles and CL on the left ovary, and lack of edema of the endometrial folds could have an interval from treatment to ovulation within 48 hours (Figure 4). Some large follicles may even ovulate within 24 hours after treatment without the mare displaying behavioral estrus.[12,17,24,25] Some mares may have large follicles (≥35 mm) at the time of treatment which regress and become replaced by a dominant follicle of the next primary follicular wave.[24] This regression can be followed by serial transrectal palpation and ultrasonographic examinations (Figures 4 and 7)

Some studies have indicated that pregnancy rates were decreased in $PGF_{2\alpha}$ induced estrous cycles when compared to pregnancy rates from spontaneous estrous cycles.[13,18,26-28] Lindeberg et al. indicated that $PGF_{2\alpha}$ treatment may exert a negative influence on large diestrus follicles, however, it was also indicated that pregnancies doubled in $PGF_{2\alpha}$ induced cycles when the ovaries contained small follicles (<30mm) at the time of treatment.[18] Nielsen et al. indicated that only the mares in their project showing poor estrous signs were treated with $PGF_{2\alpha}$ indicating these mares may have had ongoing reproductive irregularities.[26] Cuervo-Arango et al. found pregnancy rates and embryo recovery rates strongly correlated with an increased interval of treatment with $PGF_{2\alpha}$ to ovulation[29] while Agnew et al. found administration of $PGF_{2\alpha}$ to mares with large diestrus follicles appeared to have lower pregnancy rates when compared to mares bred on spontaneous estrus cycles.[28] In contrast to these studies, Metcalf and Thompson found no difference in pregnancy rates when comparing $PGF_{2\alpha}$ induced estrous cycles and spontaneous estrous cycles.[30]

Clinically, it can be challenging to know if a diestrus follicle ≥35mm is viable. In addition to the studies mentioned previously, studies by Samper et al.[24] and Burden et al.[12] demonstrate that mares with larger diestrus follicles can develop uterine edema within 24 hours, and the development of uterine edema is a strong indicator of follicle viability.

Ultimately, more research is needed in this area to determine the fertility of large (>35 mm), diestrus follicles. The ability to more accurately manage mares with diestrus follicles will increase breeding season efficiency.

Estrus/ovulation synchronization programs

The purpose of estrus/ovulation synchronization can vary from timed insemination to nuclear transfer. Regardless of the purpose of the synchronization, the goal is to align the ovarian activity of a group of mares in order to synchronize both estrus and ovulation. Older protocols, such as the use of $PGF_{2\alpha}$ and progesterone, can be effective at synchronizing estrus, but fail to synchronize ovulation, leaving a variable time interval from the end of treatment to ovulation of the mares in the synchronization protocol, while newer protocols, such as progesterone, estrogen, $PGF_{2\alpha}$, and an ovulation induction agents in combination, are effective in synchronizing both estrus and ovulation. It is important to consider what is occurring in the ovary with each of the techniques/protocols used in order to better understand the expected outcome. The following paragraphs will describe some of the older and newer protocols used for estrus/ovulation synchronization.

Prostaglandin $F_{2\alpha}$ can be and is used in mare estrus synchronization programs although it is rarely used as the sole method of synchronization.[31] In the instance of a single timed insemination or the single client desiring to shorten the diestrus period, the practitioner can attempt to inform the client of the expected interval of treatment to ovulation without knowing the follicular status of the ovaries. However, if it is not known whether the mare has only one primary follicular wave, or multiple follicular waves (see Figure 1), giving the client the typical time period of three to five days for return to estrus after treatment could potentially cause frustration. Based on the information discussed in the previous section, a mare

can fall into any of the described scenarios and have a wide range of interval from treatment with $PGF_{2\alpha}$ to ovulation. Knowing the follicular status of the ovaries via transrectal palpation and ultrasonography can assist in giving the client a more informed estimate of when the mare should return to estrus and proceed to ovulation. For example, when presented with a mare that has a 34mm diestrus follicle and a mature CL (at least 5 days post-ovulation), as depicted in Figure 6, treatment with $PGF_{2\alpha}$ on the same day of the ultrasonographic examination would likely result in a shorter interval from treatment to return to estrus (average interval to ovulation 7.8 days[12]), whereas if a mare is presented with a diestrus follicle(s) ≤10mm and a mature CL as depicted in Figure 5, one could expect an average of 11.8 days[12] from the time of treatment to ovulation. Thus treating single mares with $PGF_{2\alpha}$ alone, where tight synchrony is not the goal of treatment, usually does not lead to frustration. However, synchronization of multiple mares with $PGF_{2\alpha}$ only can lead to a group of mares that are not closely synchronized.

Most synchronization programs utilize some form of exogenous progesterone to provide negative feedback on the adenohypophysis, suppressing luteinizing hormone (LH) secretion. After the prescribed period of time administering progesterone, $PGF_{2\alpha}$ is administered to lyse any residual luteal tissue that may be present and the mares are checked in three to five days for return to estrus.[32,33] However, the primary limitation of this estrus synchronization program is that progesterone alone only suppresses LH secretion by the adenohypophysis, not follicle stimulating hormone (FSH) secretion.[33] Therefore, maturation of the follicle is halted and ovulation can be inhibited; however, without suppression of FSH, follicular waves can and do develop, as shown in the middle and bottom panels of Figure 1 when mares are under the influence of elevated levels of endogenous progesterone. The progesterone eliminates behavioral estrus even in the face of endogenous estrogen as discussed previously; however, ovarian activity and follicular waves are not suppressed, which results in greater variability in return to estrus and ovulation after treatment. Several products are available for progesterone treatment with typical treatment regimens being daily dosing for 10 days with oral altrenogest at 0.044 mg/kg or intramuscular injection of progesterone in oil at a dose of 150 mg/day. Another injectable progesterone product is available in a slow-release formulation that maintains elevated progesterone for approximately seven days.[34] Altrenogest has also become available in an injectable slow-release formulation. Prostaglandin$F_{2\alpha}$ is typically given on day 10 of the daily oral or injectable treatment protocols or ten days after injection with the slow-release progesterone or altrenogest products with expected return to estrus in three to four days and ovulation in seven to ten days following $PGF_{2\alpha}$ treatment. The appropriate use of ovulation inducing agents, described elsewhere[32], most commonly human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH) analogs, can help tighten ovulation synchrony when used in conjunction with progesterone treatments.

Estrus synchronization programs that utilize a combination of progesterone and estradiol-17β obtain suppression of LH secretion, as described above, as well as suppression of FSH secretion from the adenohypophysis and therefore, suppression of ovarian follicular development.[35] Suppression of the ovary in this manner provides much tighter synchrony than can be achieved by progesterone alone because recruitment of follicles for follicular waves does not occur in the absence of FSH.[32] Traditionally, progesterone and estradiol-17β were mixed in vegetable oil at concentrations of 50 mg/ml and 3.3 mg/ml, respectively, and administered intramuscularly at a daily dose of 150 mg progesterone and 10 mg estradiol-17β for ten days with administration of $PGF_{2\alpha}$ on the last day of treatment.[35] In the early 1990's, research into sustained release of these hormones to alleviate the need for daily injections was conducted.[36,37] Although no Food and Drug Administration (FDA) approved products are currently available, compounded products are available with sustained release of progesterone and estradiol-17β. One injection of this long-acting/slow-release progesterone and estrogen product results in ovarian suppression occurring for approximately ten days. Prostaglandin$F_{2\alpha}$ is used at the end of the synchronization treatment in order to eliminate any luteal tissue which may still be present in the ovary. The mares are then checked three to five days after the last day of treatment to assess ovarian follicular status. The appropriate use of ovulation inducing agents, as mentioned above, will further synchronize ovulation, tightening overall synchrony of mares in the synchronization program.

Summary

In summary, treating mares with $PGF_{2\alpha}$ during the breeding season is a common practice and having a basic understanding of primary and secondary follicular waves helps the practitioner when treatment decisions must be made. The use of transrectal palpation and ultrasonography to assess ovarian follicular status can greatly augment the effectiveness and efficiency with which $PGF_{2\alpha}$ treatment can be utilized by practitioners, since the diameter of secondary follicles is inversely proportional to the time of treatment with $PGF_{2\alpha}$ to ovulation. Synchronization programs based on $PGF_{2\alpha}$ treatment alone do not provide the tight synchrony seen with other programs due to the variation of primary and secondary follicular waves experienced in the ovaries of mares. The use of progesterone in combination with estrogen, followed by treatment with $PGF_{2\alpha}$ at the end of the treatment period, and the appropriate use of ovulation inducing agents provides the tightest synchrony available. In conclusion, the optimal use of $PGF_{2\alpha}$ in mares requires an understanding of the underlying physiological events associated with follicular dynamics throughout the estrous cycle.

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(Editor's Note: Images in this manuscript appear in color in the online edition of Clinical Theriogenology.)

Table. Terminology commonly used in association with follicular dynamics in mares. Reprinted with permission from John Wiley and sons.[5]

Characteristic	Definition
Follicular wave	A cohort or group of antral follicles (mean 7 to 11) that are first detected (≥ 6 mm in diameter) within 1 to 2 days of one another and increase in diameter similarly until follicle deviation.
Follicle or wave emergence	The earliest ultrasonic detection of follicles (≥ 6 mm) of a wave compatible with tracking individual growing and regressing follicles in a reliable and consistent manner. Emergence of a wave is a useful reference point for aligning the emergence of waves that occur at different times among mares.
Common growth phase	The period from emergence of a major follicular wave to beginning of deviation (6 to 7 days). All follicles of the wave grow at a similar rate (about 3 mm/day) during the common growth phase.
Follicle deviation	The dissociation in diameter between the largest follicle and next largest follicles of a major wave that begins, on average, when the largest follicle reaches 22.5 mm. The largest follicle continues to grow whereas the next largest follicles cease growth and regress after the beginning of deviation. The beginning of deviation is calculated to occur at the ultrasound examination preceding the first examination with an apparent change in diameter difference between two largest follicles. The beginning of deviation is a useful reference point for aligning the growth profiles of follicles of a wave for comparing structural and functional characteristics within and among mares.
Dominant follicle	The largest follicle of a major wave that continues growth after deviation, reaches ≥ 28 mm and becomes ovulatory or anovulatory.
Subordinate follicles	The next largest follicles of a major wave relative to a dominant follicle, that cease growth after deviation, reach < 28 mm and eventually regress.
Dominance phase	The period from the beginning of deviation until the beginning of the pre-ovulatory phase (approximately 7 to 8 days). Growth of all subordinate follicles of the wave ceases during this period and typically no new follicular waves emerge.
Pre-ovulatory phase	The period beginning when the dominant follicle of the major primary wave attains ≥ 30 mm and until ovulation or formation of a hemorrhagic follicle. The pre-ovulatory follicle attains the developmental capacity to respond to an endogenous or exogenous ovulatory stimulus.
Major follicular wave	A follicular wave that develops at least one dominant follicle (≥ 28 mm) and corresponding subordinate follicles (< 28 mm) during the transitional period, estrous cycle, and early pregnancy.
Minor follicular wave	A follicular wave that does not develop a dominant follicle. Instead, the largest follicle of the wave reaches < 28 mm during the transitional period, estrous cycle, and early pregnancy.
Primary follicular wave	A major follicular wave that emerges during late diestrus where the dominant follicle becomes pre-ovulatory and results in the primary ovulation at the end of estrus.
Secondary follicular wave	A major wave that emerges during late estrus of the previous estrous cycle or early diestrus where the dominant follicle either is anovulatory and regresses or forms an anovulatory hemorrhagic follicle or results in secondary ovulation during mid-diestrus.

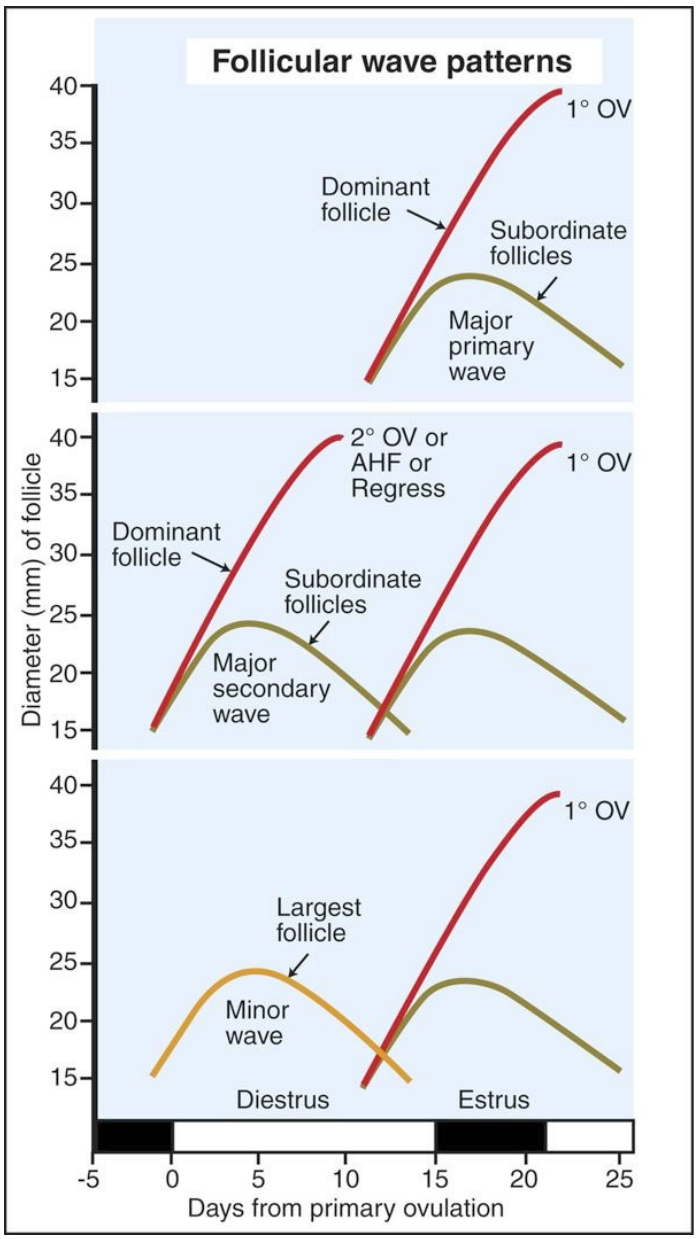


Figure 1. Various profiles of follicular wave patterns during the equine estrous cycle depicting ovulation (OV) of the dominant follicle (≥30 mm) of a primary major wave at the end of estrus and OV or anovulation and formation of a hemorrhagic follicle (AHF) or re regression of the dominant follicle of a secondary major wave during diestrus. Alternatively, either minimal follicular growth (<15 mm) or a minor follicular wave (largest follicle, <30 mm) precedes the primary major wave. Reprinted with permission from Elsevier.[6]

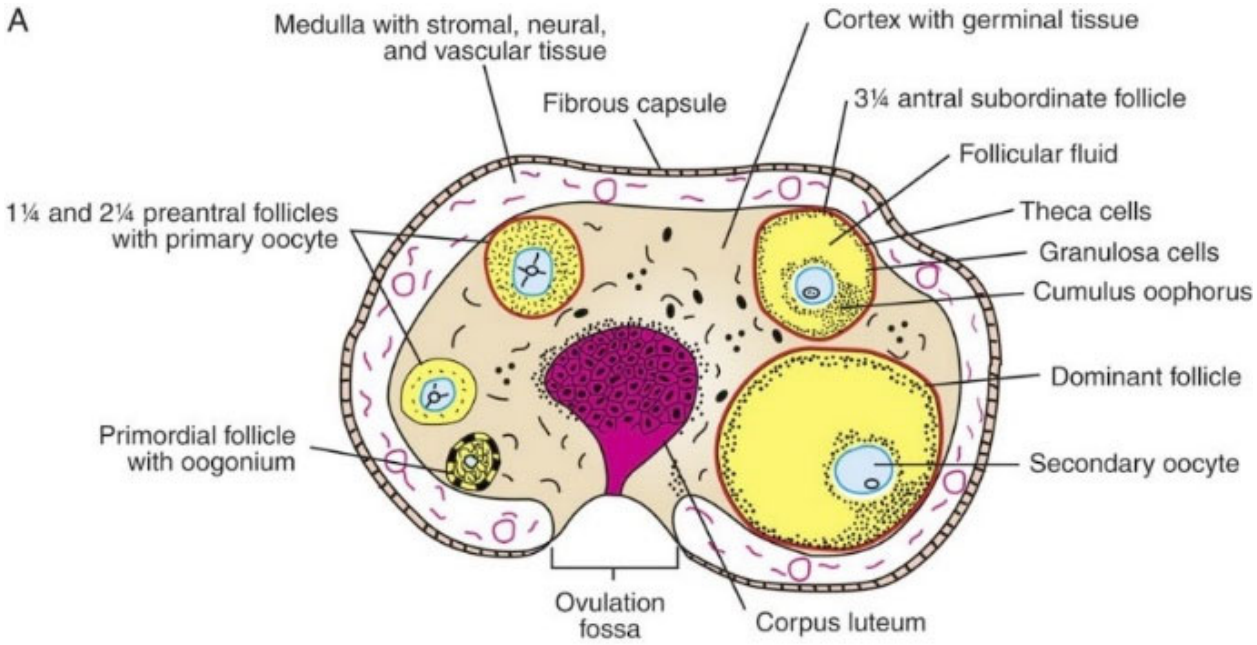


Figure 2. The morphological characteristics of the adult equine ovary (A) during the ovulatory season. Note the sequential transformation of a primordial follicle to a dominant follicle and corpus luteum following ovulation. Reprinted with permission from Elsevier.[6]

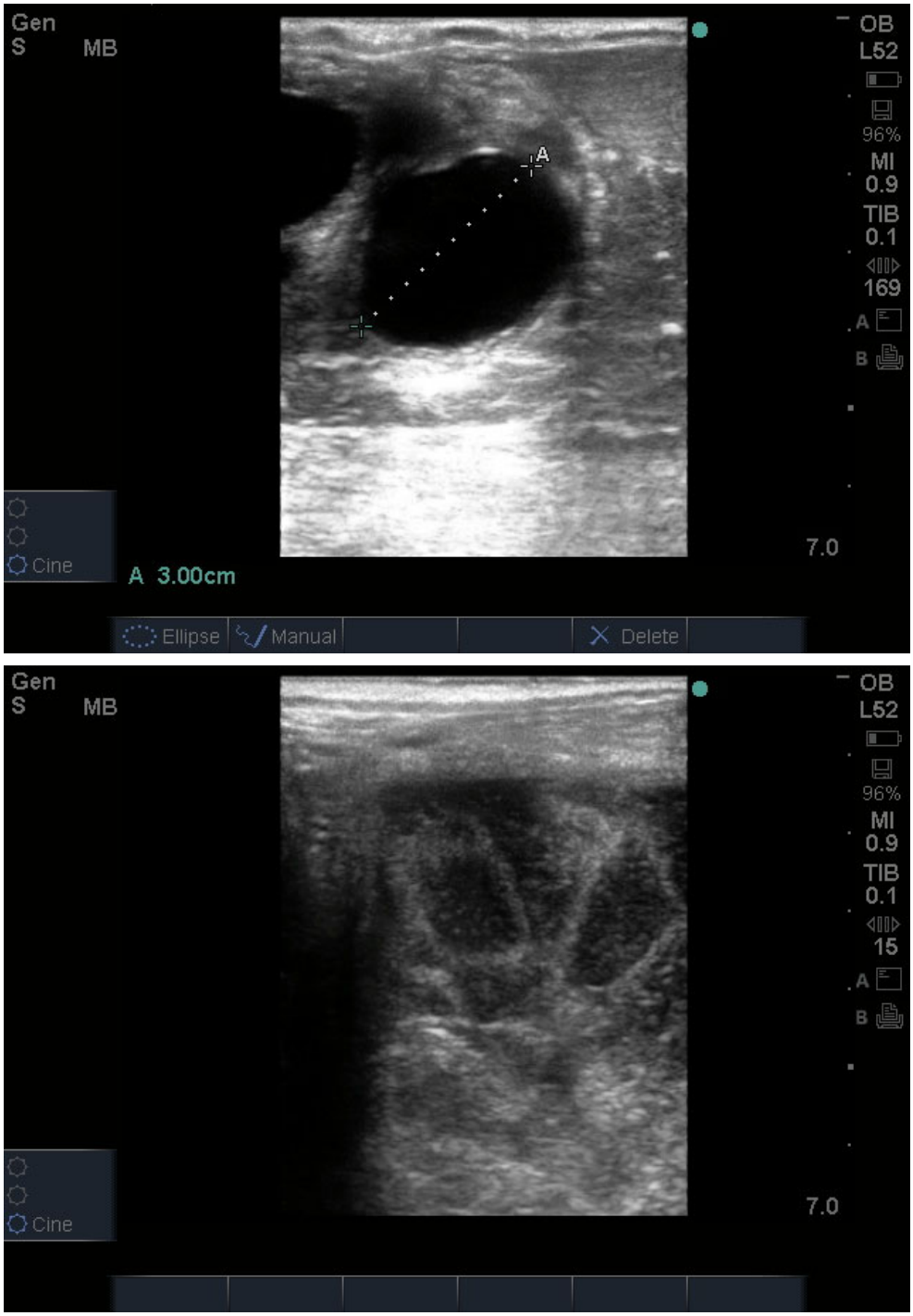


Figure 3. Top - Ultrasound image of an ovary 16 days after ovulation with a dominant follicle.
Bottom - An ultrasound image from the same mare demonstrating her uterus with edema of the endometrial folds.
Tic marks on the right side of the images equal 10 mm.

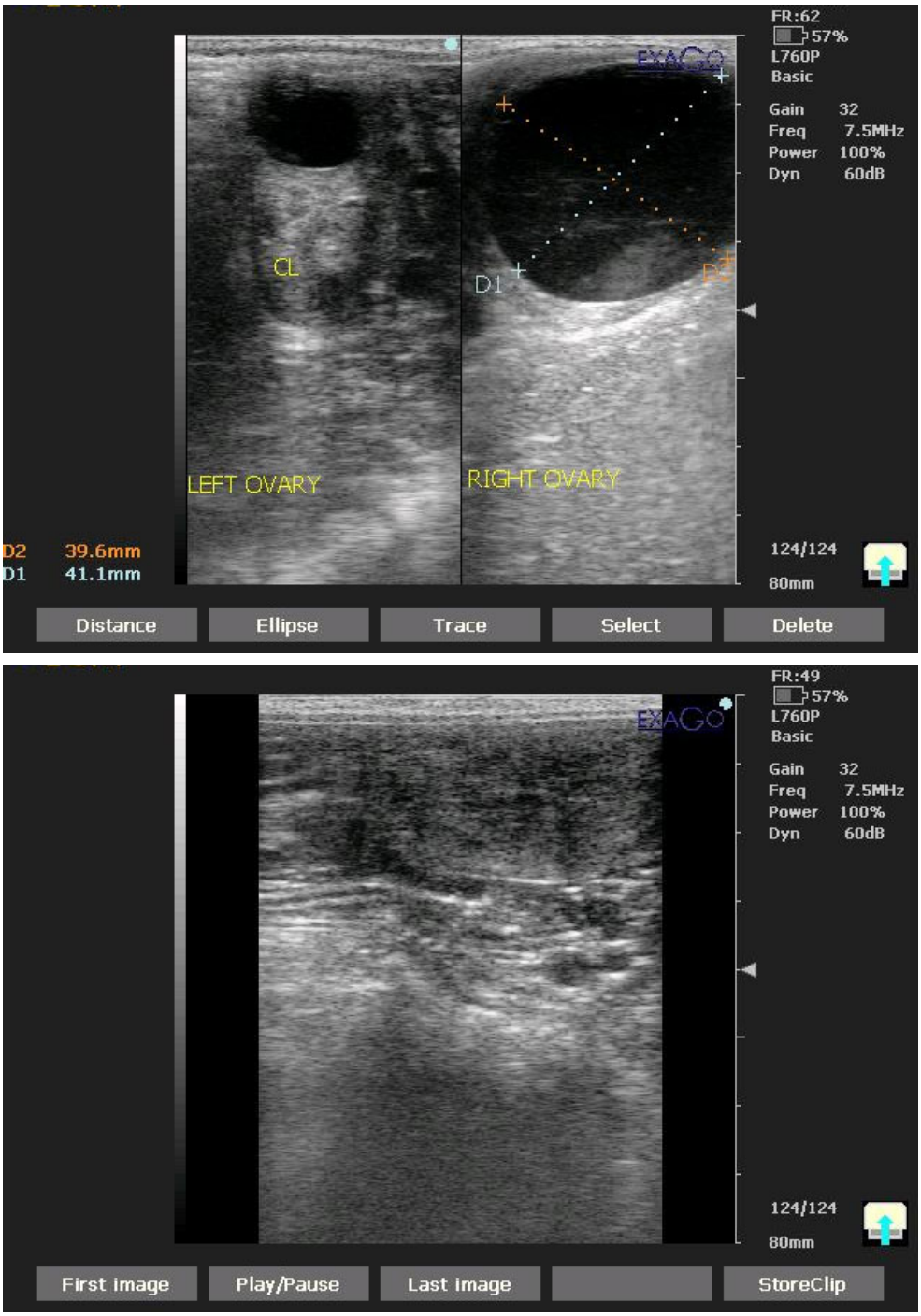


Figure 4. The top image displays a CL and small follicle on the left ovary and a large secondary follicle on the right ovary 7 days after ovulation. The bottom image is the uterus from the same mare, displaying no edema within the endometrial folds. Tic marks on the right side of the image equal 10 mm.

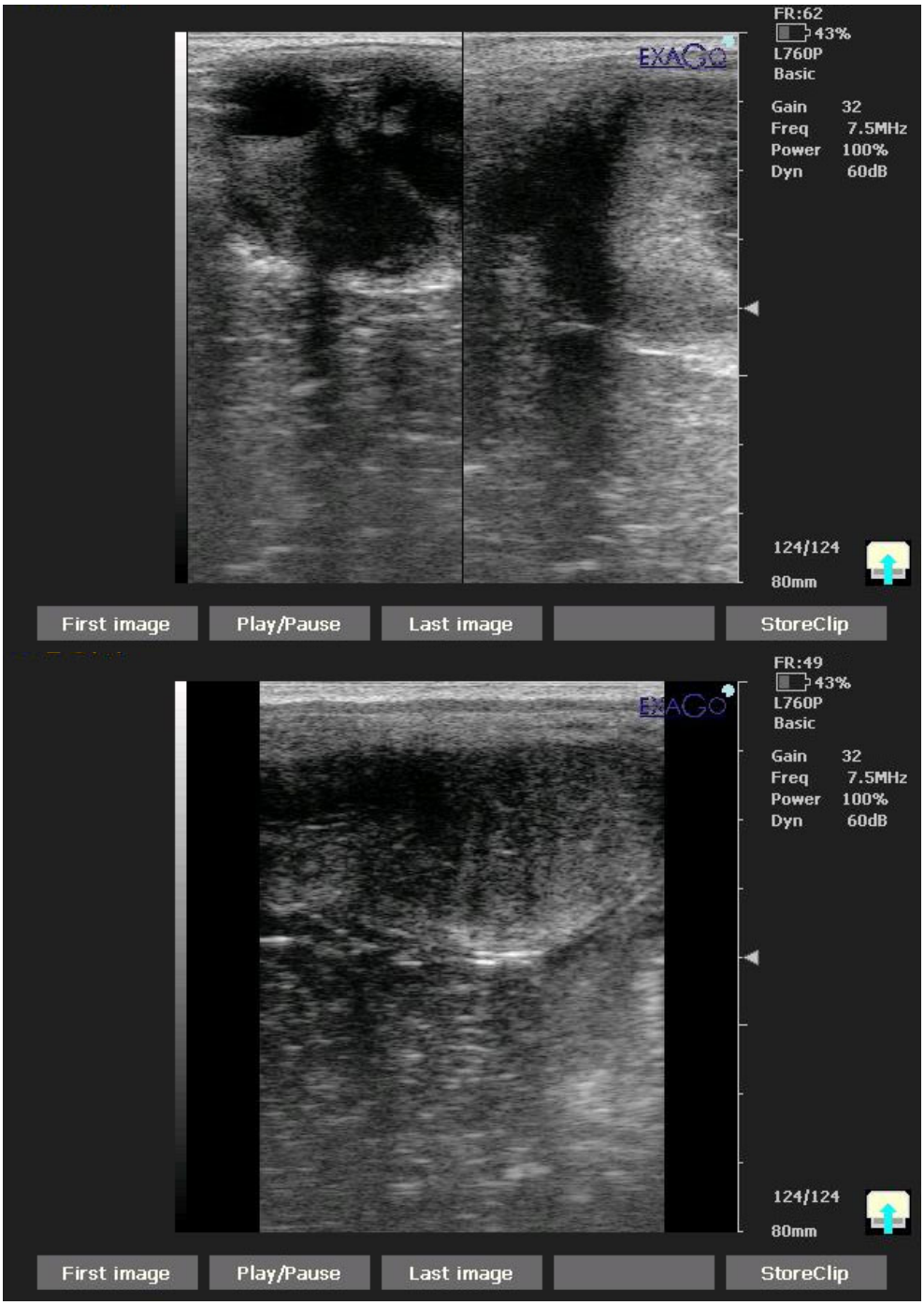


Figure 5. The top-left image shows multiple small follicles, all roughly 10 mm in diameter in the left ovary with a CL in the right ovary (top-right) 3 days after ovulation. The bottom image shows the uterus without edema of the endometrial folds. Tic marks on the right side of the images equal 10 mm.

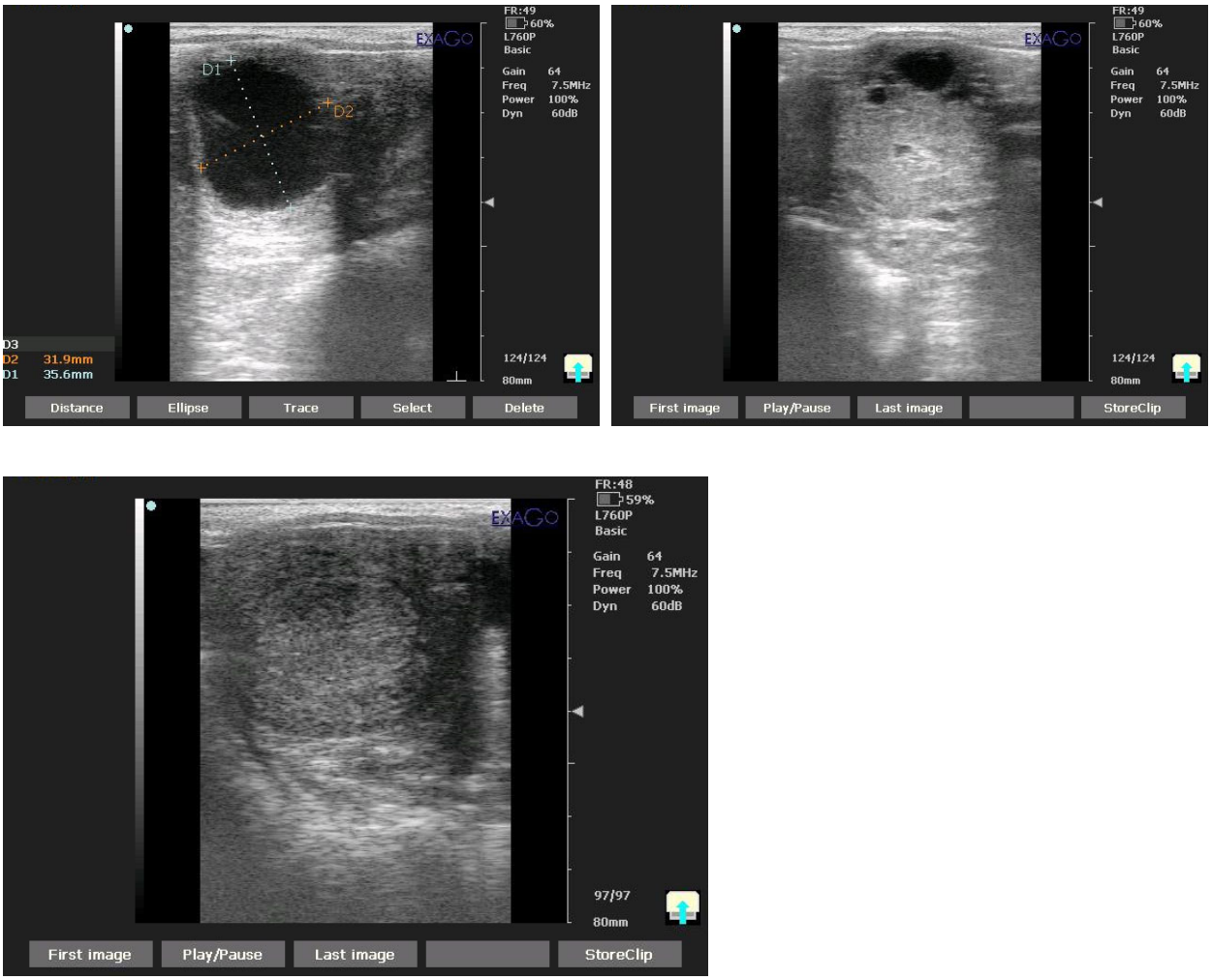
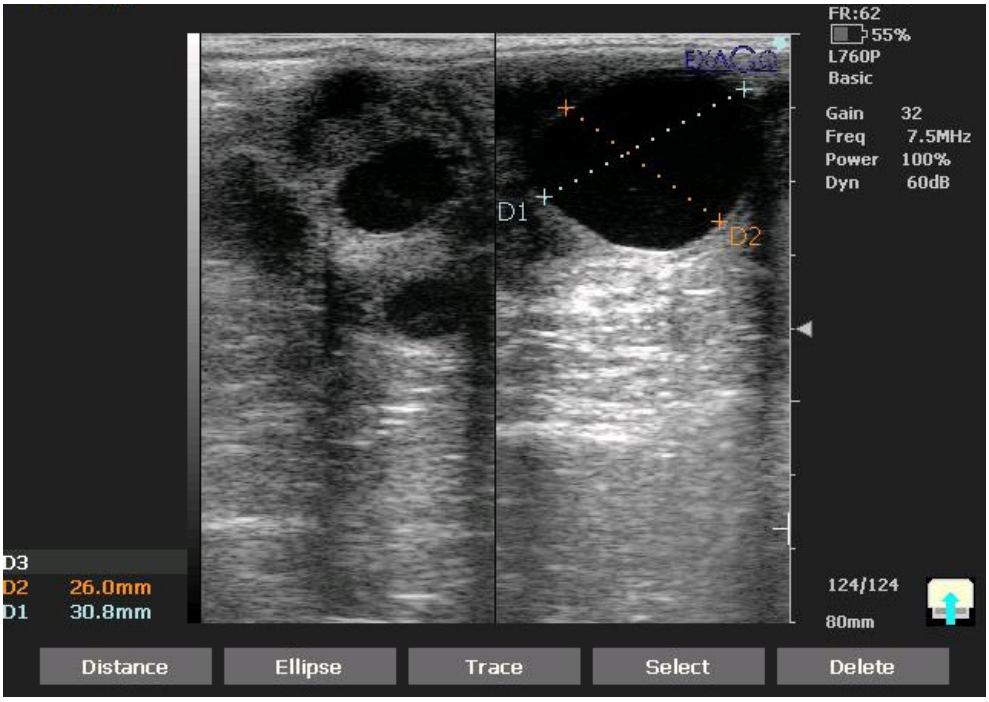


Figure 6. The top-left image shows the left ovary with a roughly 34mm follicle. The top-right image shows the right ovary with a corpus luteum. The bottom image is of the uterine horns from this mare showing no edema of the endometrial folds. This mare is 9 days after ovulation. Tic marks on the right side of the images equal 10 mm.



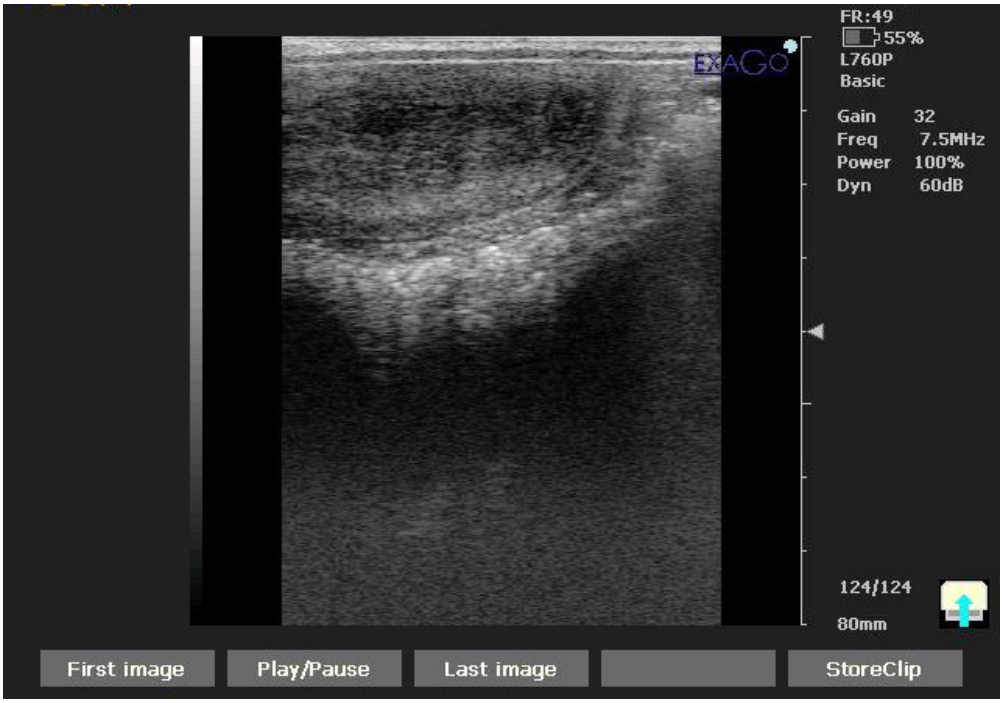


Figure 7. These images are from the same mare as Figure 4, 6 days after Figure 4 was obtained (13 days after ovulation). The CL in the left ovary has regressed, the follicle in the right ovary has regressed, and no edema of the endometrial folds is present. Tic marks on the right side of the images equal 10 mm.