Quantitative marker of Equine progesterone

Vcheck eProgesterone

BIONOTE Marketing team

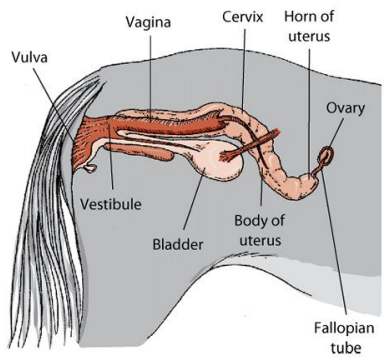
MAR 2023

Mare Reproductive Physiology

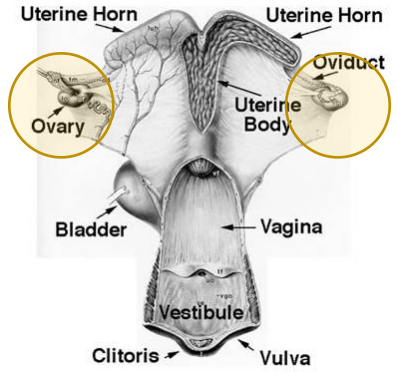
An equine ovary is unique in its internal structure.

- ✓ The equine ovary has the opposite arrangement. #5
  - The medullary (vascular) zone: superficial
  - The cortical zone: contains the oocytes and follicles is partially within the interior of the gland

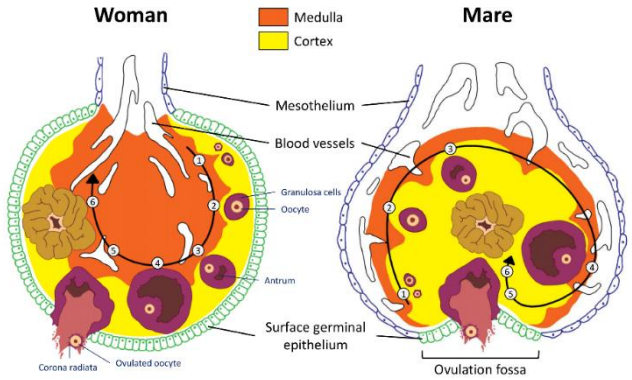
- ✓ The mare's ovaries are larger than those of other domestic species.
  and vary considerably in size during the year depending on the number and size of follicles on their surface.
  i.g. walnuts size (reproductive quiescence) ➔ a large orange size (cyclic season when there is a 50-mm follicle on the ovary)



The reproductive system of a mare #6



Frontal view #4



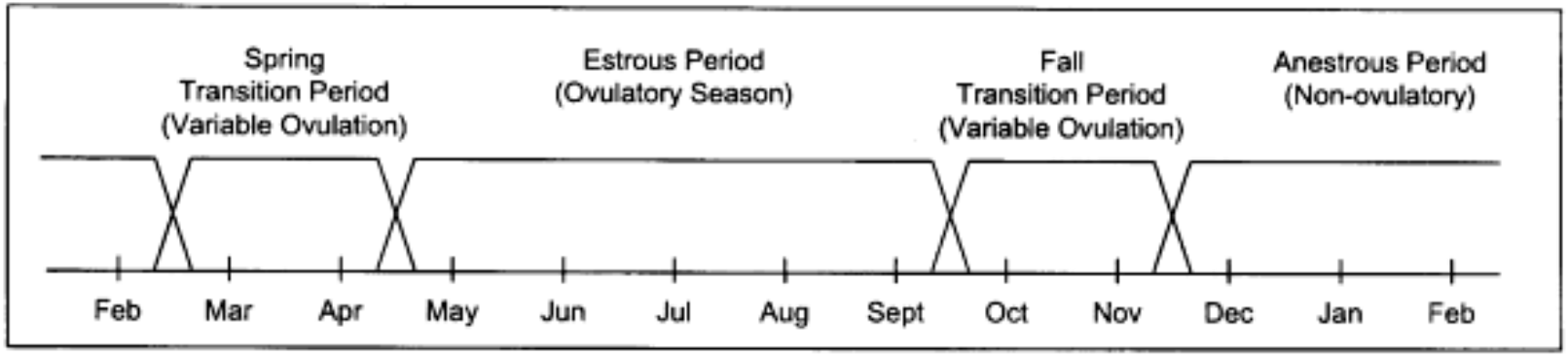
Comparative ovarian anatomy and folliculogenesis in woman and mare #9

Mare Reproductive Physiology

The mare has a “seasonally polyestrous” type of estrous cycle.

- ✓ She undergoes regular estrous cycles during a portion of the year (mid-April to mid-September) #15
- ✓ During winter, most mares show no behavioral signs of sexual receptivity and fail to develop follicles that ovulate (in the “winter anestrus” period). - In the northern hemisphere –
- ✓ This is nature's way of preventing the arrival of a foal during bad weather. #4
  (The average gestation length: 340 days ±20, post-breeding #8)

Figure 1. Mare seasonality.



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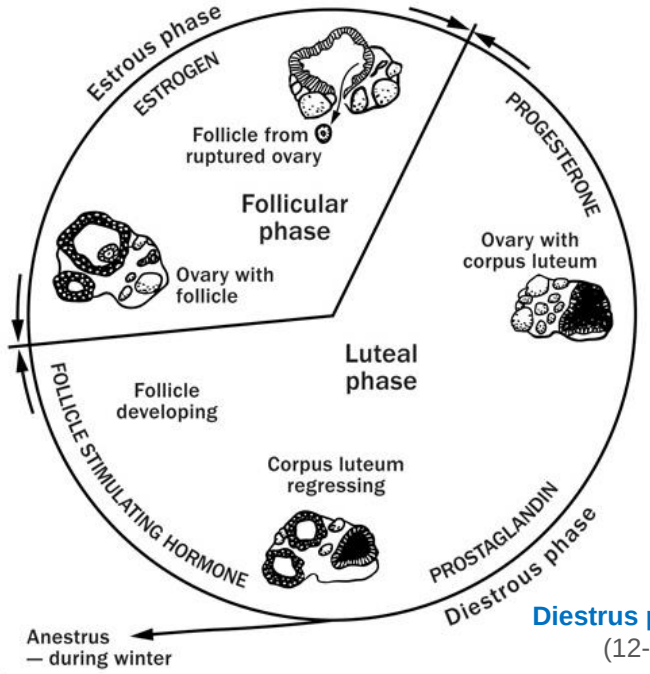
Mare Reproductive Physiology

The estrous cycle (21 days) is divided into two physiological parts—estrus and diestrus

1) **The estrus phase (in heat)**: 6 days (4-10 days)
   - The time of follicular maturation and ovulation
   - **Ovulation**: most frequently occurs 24 to 48 hours prior to the end of estrus
     ➔ Breeding should occur within 12 hours of ovulation.

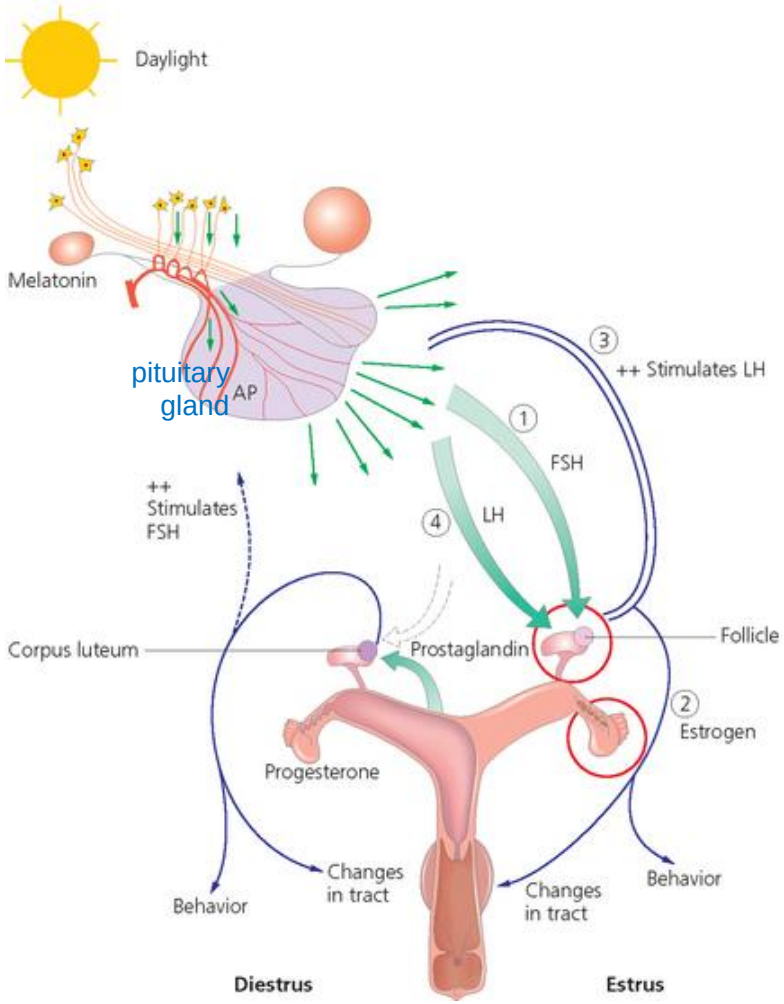
2) **The diestrus phase (out of heat)**: 15 days (12-18 days)
   - The follicle that ovulated at the end of estrus develops into a structure called the **corpus luteum (CL)**

Estrus phase 6 days
(4-10 days)



Diestrus phase 15 days
(12-18 days)

Mare Reproductive Physiology



Hormonal control of the estrous cycle in the mare. #5

Hormones in the "Estrus phase" (in heat, 6 days) #8

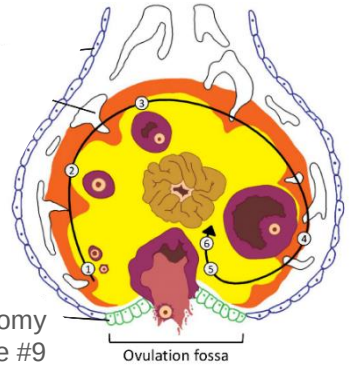
As spring approaches, the pituitary gland is stimulated by increased daylight to enhance follicle-stimulating hormone (FSH) production.

① **FSH** travels to the ovaries to initiate the development of a follicle. The developing follicle produces estrogens.

② When blood **estrogen** reaches a certain level, a surge of luteinizing hormone (LH) is released from the pituitary gland into the bloodstream.

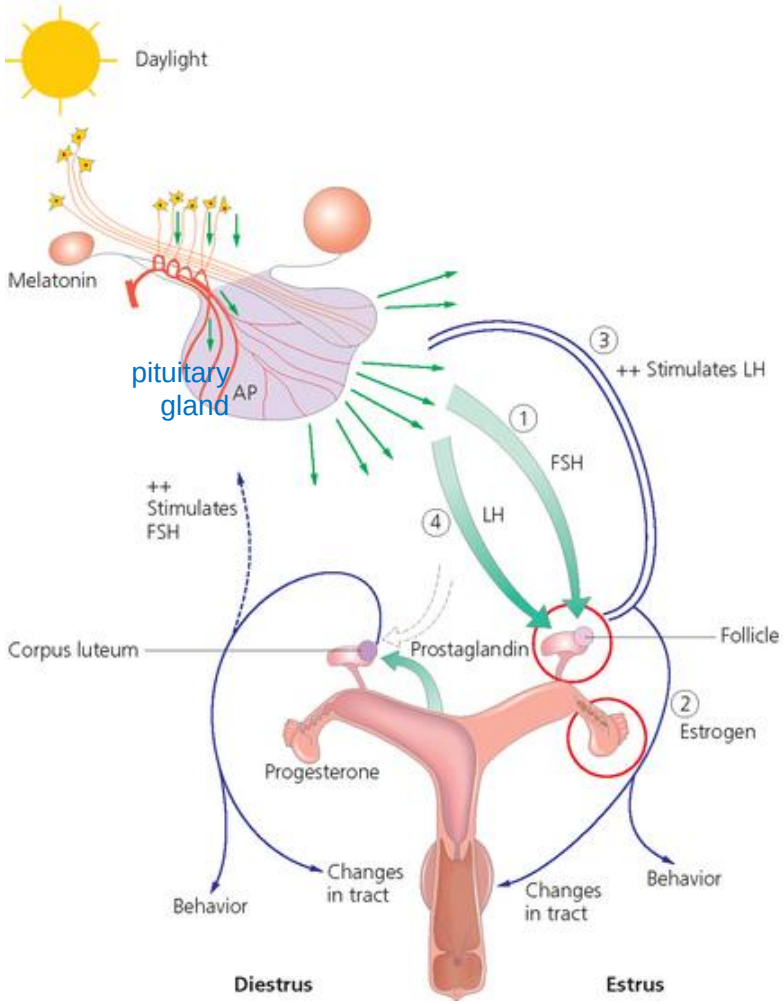
③ **Estrogens** are responsible for the clinical signs of estrus and act on the oviducts, uterus, and cervix to prepare the reproductive tract for pregnancy.

④ The **surge of LH** causes the follicle on the ovary to rupture, resulting in ovulation. The cavity left by the ruptured follicle becomes the corpus luteum, sometimes called the yellow body.



Ovarian anatomy in mare #9

Mare Reproductive Physiology

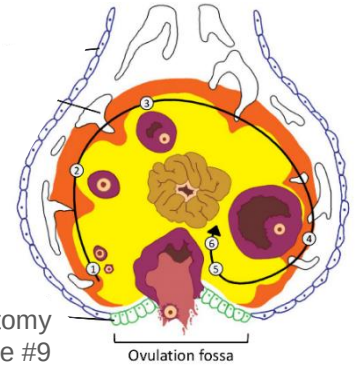


Hormonal control of the estrous cycle in the mare. #5

Hormones in the "Diestrus phase" (out of heat, 15 days) #8

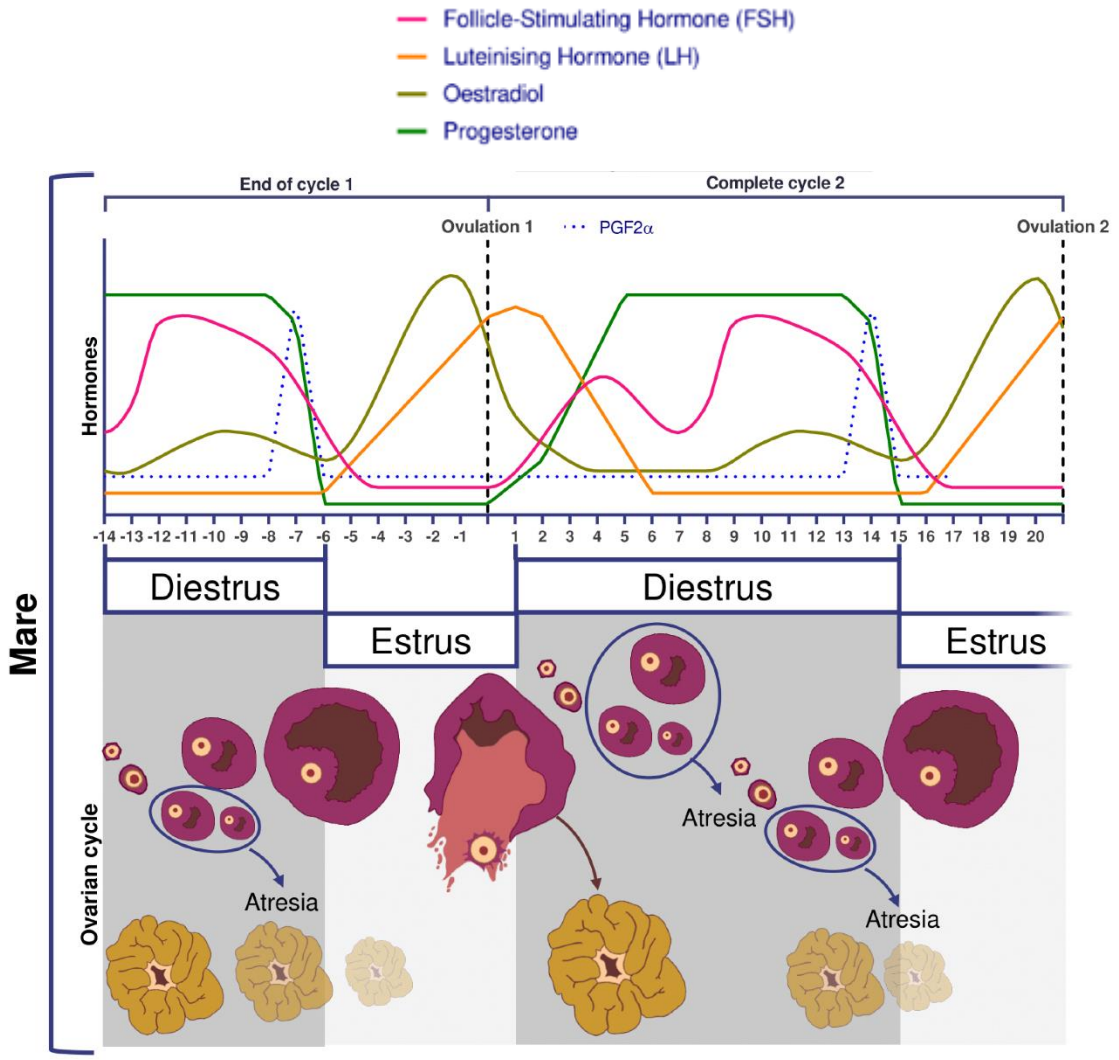
As the corpus luteum develops, it starts to produce progesterone.

- The feedback of progesterone via the bloodstream inhibits the release of LH. Under the influence of progesterone, the mare will not show estrus.
- **Progesterone's function is to maintain the pregnancy** by maintaining a uterine environment conducive to fetal development.



Ovarian anatomy in mare #9

Mare Reproductive Physiology



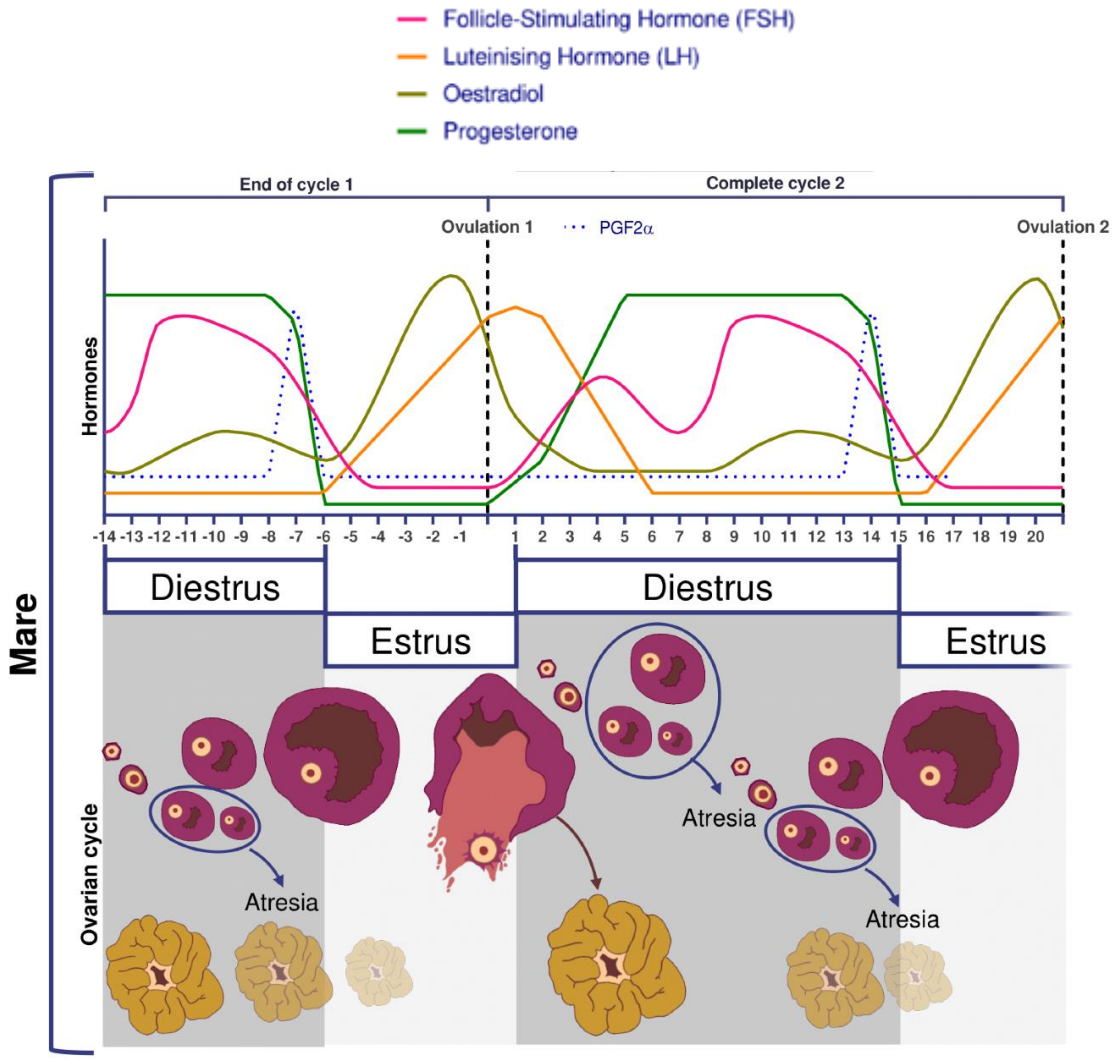
Ovarian and hormonal cycles in mares. #9

If the mare does not conceive, #8

the corpus luteum remains functional for about 12-14 days.

- ➔ At this time, prostaglandin is released from the endometrium.
- ➔ Prostaglandin has a **luteolytic effect** - it acts on the corpus luteum via the bloodstream, causing it to regress.
- ➔ As the corpus luteum regresses, progesterone levels are reduced, resulting in the removal of the inhibition of LH secretion.
- ➔ The cycle starts over again.

Mare Reproductive Physiology



Ovarian and hormonal cycles in mares. #9

If the mare conceives, #8

hormonal activities are essentially the same as for the 12-14 days post-ovulation.

- ➔ Pregnancy recognition is stimulated by the action of the developing embryo migrating throughout the uterus.
- ➔ This action inhibits prostaglandin release, resulting in an anti-luteolytic effect.
- ➔ So, the corpus luteum remains functional, progesterone levels are maintained and the pregnancy is continued. (“**Primary corpus luteum**”)

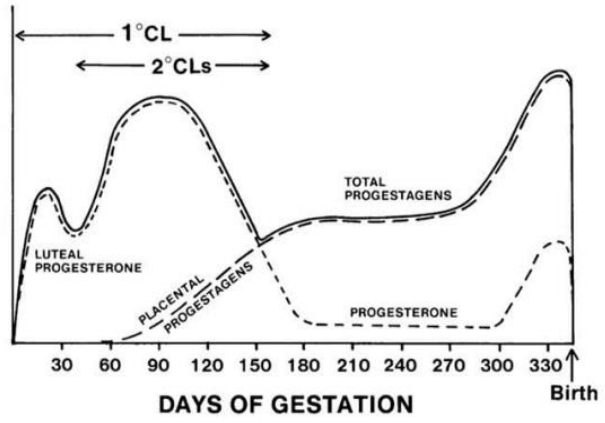
Mare Reproductive Physiology

Primary corpus luteum (Day 0 to Day 130-150)

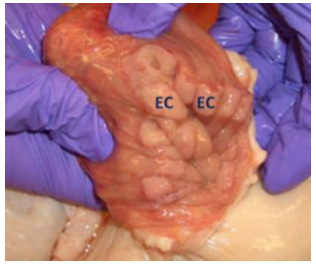
- Progesterone concentration: as low as 2.5 ng/mL, somewhat higher concentrations between 4 and 10 ng/mL. #2
- At day 35–40 of gestation, the corpus luteum starts to regress, resulting in declining blood progesterone levels. #11

Secondary corpus luteum (Day 70 to Day 150)

- However, a unique feature of the equine allantochorionic placenta is the formation of endometrial cups, secreting the hormone equine chorionic gonadotropin (eCG).
- Due to its expression of LH-like and FSH-like biological activity, secondary follicles from follicular waves luteinize or ovulate.
- These secondary corpora lutea will be responsible for a rise in and maintenance of maternal serum progesterone.



Equine gestation hormones. Courtesy of Dr. D.P. Neeley. #11

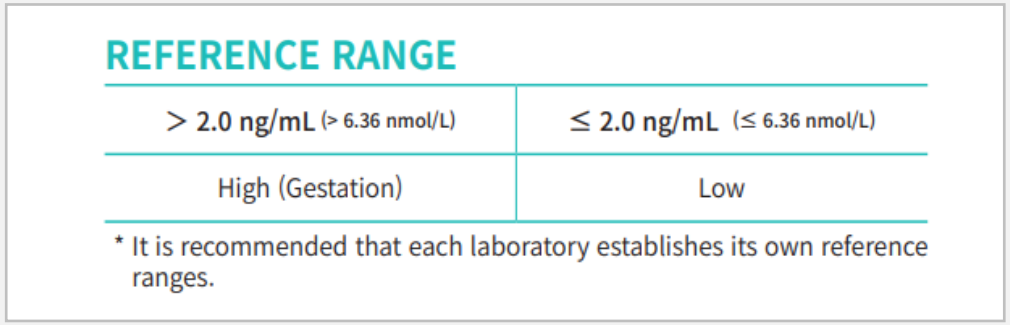


Equine endometrial cups (EC) #22

Clinical utility of Progesterone

Progesterone is …

- ✓ Responsible for the suppression of behavioral estrus, closure of the cervix, alterations in endometrial glandular function, and other physiological events.
  It is also the most important hormone for the maintenance of early pregnancy to day 45 in the mare. #2, 12
- ✓ Progesterone is required for early embryo survival. #13
  : A progesterone concentration of 2 ng/mL is considered the minimum endogenous amount necessary to support pregnancy. #2
  - **< 2 ng/ml** : associated with embryonic loss ➔ P4 supplementation is considered #19
  - **> 4 ng/ml** : a higher rate of embryonic survival (considered to be adequate to maintain pregnancy) #13

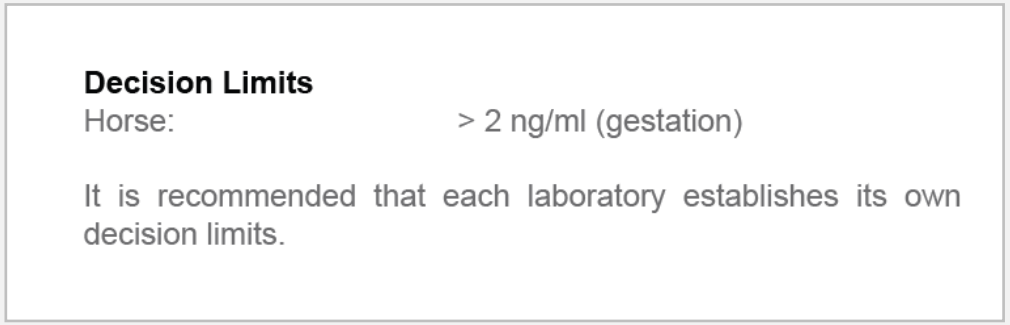


REFERENCE RANGE

> 2.0 ng/mL (> 6.36 nmol/L)	≤ 2.0 ng/mL (≤ 6.36 nmol/L)
High (Gestation)	Low

* It is recommended that each laboratory establishes its own reference ranges.

Reference range of Vcheck eProgesterone (insert)



Decision Limits

Horse: > 2 ng/ml (gestation)

It is recommended that each laboratory establishes its own decision limits.

Reference range of Eurolyser equine progesterone (insert)

Clinical utility of Progesterone

Administration of supplemental hormones

There is a lack of scientific evidence supporting exogenous progesterone supplementation as a means of improving pregnancy maintenance in mares.
However, this continues to be a common practice in broodmare reproduction. #13

1) **When there are suspicions of inadequate progesterone production,**
   (with suspected luteal insufficiency)

2) **When facing mares that repeatedly fail to conceive or carry a pregnancy to term,**
   (in case of endotoxemia and of stressful events)

Progestogen supplementation can be used as treatment for early-bred or known pregnant mares if the mare's endogenous progesterone production is suspected of being low. #2

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Clinical utility of Progesterone

Administration of supplemental hormones

Options for therapy include the administration of natural progesterone or synthetic progestin (altrenogest). #12 (**starting on day 3 after ovulation and continuing until 100–120 days of pregnancy**) #20

- **Altrenogest** (allyltrenbolone) : a synthetic progestin widely used in equine reproduction
  - The label dose (0.044 mg/kg SID, oral)
  - Note that altrenogest does not cross-react with progesterone assays; therefore endogenous progesterone production may be monitored while mares are supplemented with this product.

Regu-Mate Solution (**altrenogest**)

- **Natural progesterone** :
  - The label dose (150 mg/day, IM)
  - If supplementing with parenteral natural progesterone, a serum progesterone determination will show the cumulative effect of both the exogenously administered product and endogenous production by the mare. #14

NexGen Progesterone

Clinical utility of Progesterone

Administration of supplemental hormones

Options for therapy include the administration of natural progesterone or synthetic progestin (altrenogest). #12 (**starting on day 3 after ovulation and continuing until 100–120 days of pregnancy**) #20

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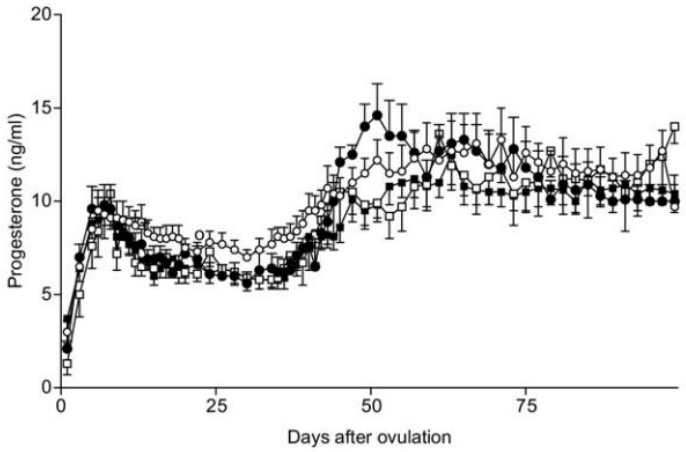

Mean concentration of progesterone
In early pregnant mares aged 4 to 8 years #21

Clinical utility of Progesterone

In Non-pregnant Mares,

The analysis of progesterone levels is a useful guide to diagnosis and treatment in the acyclic or irregularly cyclic mare.

- ✓ Mare blood progesterone concentrations indicate the presence or absence of luteal tissue and are not diagnostic for pregnancy. #17
- ✓ Progesterone concentrations less than 1 ng/ml at 18 to 21 days after ovulation suggest that a mare is not pregnant.
- ✓ In the non-pregnant mare, **progesterone concentrations > 2 ng/ml indicate functional luteal tissue** and suggest that prostaglandin treatment should induce luteolysis, providing that the corpus luteum is more than 4 days old. #18

Clinical utility of Progesterone

Clinical Applications

: Progesterone assay is a simple and useful method of assessment of the presence of a functional corpus luteum, producing progesterone hormones

In pregnant mares,

1) To evaluate the maintenance of early pregnancy, (Day 21~45)

progestogen supplementation can be considered as a means of improving pregnancy maintenance

A. When there are suspicions of inadequate progesterone production,
(with suspected luteal insufficiency)

B. When facing mares that repeatedly fail to conceive or carry a pregnancy to term,
(in case of endotoxemia and of stressful events)

2) To monitor endogenous progesterone production in mares treated with supplemental hormones

In non-pregnant mares,

1) To diagnose and treat the acyclic or irregularly cyclic mare (functional luteal tissue) (Day 21~)

Mare Reproductive Physiology

Detection of Estrus

- ✓ Rental palpation and teasing are the two most common management tools used in the detection of heat.
  - Rectal palpation: monitoring of parameters of follicular size, cervical size and consistency, and uterine tone (i.g. A mare with a large, very soft follicle that has an open cervix is a prime candidate for breeding.)
  - Teasing: behavioral signs of estrus include winking of the vulva, urination, squatting, and seeking the stallion
- ✓ Increasingly, ultrasonography is being utilized for estrus detection and determination of ovulation
  - Ultrasonography: follicular size, early ovulation, uterine changes, and abnormalities of the reproductive tract

Breeding in Mares

- ✓ Ideally, to maximize the chance of conception, breeding should occur within 12 hours of ovulation.
- ✓ Breeding or insemination of mares, starting on Day 2 or 3 of estrus and continuing every other day throughout the estrus, is a practical means of achieving satisfactory pregnancy rates.

Rectal palpation

Pen teasing #16

Mare Reproductive Physiology

Pregnancy Determination

Several management techniques are used for pregnancy determination. #15

- ✓ To watch for signs of heat: one of the simplest ways
  - A mare may be pregnant if she exhibits no signs of heat 18 to 20 days after her last ovulation.
- ✓ Rental palpation
  - Mares are most often rectally palpated 18 to 45 days following the last day of insemination
- ✓ Ultrasonography: has gained attention for use in pregnancy determination
  - Normally used following the 14th-day post-breeding, but it can detect pregnancies as early as 10 days post-ovulation

Reference: #1. Equine Female Reproductive Testing. Animal Health Dianostic Center, Cornell University College of Veterinary Medicine #2. Equine Pregnancy and Clinical Applied Physiology. 2013, vol. 59, AAEP proceedings #3. Clinical Veterinary Advisor: The Horse. 2012, Page 956 #4. Dr. Jack Sales, DVM. Equine Reproduction. https://www.horsecoursesonline.com/college/repro/lesson_two_319.htm #5. Michelle LeBlanc, Cheryl Lopate, Derek Knottenbelt and Reg Pascoe. THE MARE. https://veteriankey.com/the-mare/ #6. The Gonads and Genital Tract of Horses. 2022, Merek manual. https://www.merckvetmanual.com/horse-owners/reproductive-disorders-of-horses/the-gonads-and-genital-tract-of-horses #7. Ovarian Follicular Dynamics During the Estrous Cycle in the Mare. Israel Journal of Veterinary Medicine, Vol. 67 (1) #8. Anatomy, physiology and reproduction in the mare. 2010, https://www.ontario.ca/page/anatomy-physiology-and-reproduction-mare #9. Benammar A, Derisoud E, Vialard F, Palmer E, Ayoubi JM, Poulain M, Chavatte-Palmer P. The Mare: A Pertinent Model for Human Assisted Reproductive Technologies? Animals. 2021; 11(8):2304. #10. Michelle LeBlanc, Cheryl Lopate and Derek Knottenbelt. PREGNANCY. https://veteriankey.com/pregnancy/ #11. Staging and Prediction of Parturition in the Mare. Clin Tech Equine Pract 4:219-227. #12. Dr. Dave Scofield. Progesterone Therapy for Broodmares: Part 1. https://info.selectbreeders.com/blog/progesterone-therapy-for-broodmares-part-1 #13. Canisso IF, Beltaire KA, Bedford-Guaus SJ. Premature luteal regression in a pregnant mare and subsequent pregnancy maintenance with the use of oral altrenogest. Equine Vet J 2013;45:97–100. #14. Jackson SA, Squires EL, Nett TM. The effect of exogenous progestins on endogenous progesterone secretion in pregnant mares. Theriogenology 1986;25:275–279. #15. Elisabeth Giedt, Kris Hiney. Reproductive Management of the Mare. 2019. https://extension.okstate.edu/fact-sheets/reproductive-management-of-the-mare.html #16. Dr. IFFAT KAWSAR, 8 Most Common Methods of Horse Teasing For Detection of Heat. https://www.thevetexpert.com/8-most-common-methods-of-horse-teasing-for-detection-of-heat/ #17. Pregnancy Evaluation in the Mare. PATRICIA L. SERTICH, in Current Therapy in Large Animal Theriogenology (Second Edition), 2007 #18. Submission of laboratory samples and interpretation of results. In Diagnostic Techniques in Equine Medicine (Second Edition), 2009 #19. Ousey JC. Hormone profiles and treatments in the late pregnant mare. The Veterinary Clinics of North America. Equine Practice. 2006;22(3):727-747. #20. Satué, K., & Carlos Gardon, J. (2021). Physiological and Clinical Aspects of the Endocrinology of the Estrous Cycle and Pregnancy in Mares. Animal Reproduction in Veterinary Medicine. #21. Willmann C, Schuler G, Hoffmann B, Parvizi N. Effects of age and altrenogest treatment on conceptus development and secretion of LH, progesterone and eCG in early-pregnant mares. Theriogenology. 2011;75:421-428. #22. IL-22 Is Expressed by the Invasive Trophoblast of the Equine (Equus caballus) Chorionic Girdle. The Journal of Immunology 188(9):4181-7