



Diestrus: This period occurs after the ovulation. After ovulation, progesterone concentration increases rapidly to maximal values (usually > 10ng/mL), remains high during luteal phase and declines rapidly following regression of the corpus luteum. The length of diestrus is 14-15 days. During diestrus, the mare is not receptive to the stallion.

Transitional period: During transition period the mare may not have a regular estrus cycle. A few mares may have persistent corpus luteum that produces progesterone preventing estrus. Determination of progesterone levels would differentiate a mare in anestrus from one with persistent corpus luteum producing progesterone.

Anestrus: Anestrus is the time period where reproductive cyclicity stops and occurs during the winter months in horses. This period is characterized by the absence of estrus cycle and by a low progesterone level (< 1 ng/mL).

C. SAMPLING

To Detect the Onset of Cycling: Perform the analysis every 3 or 4 days and test each sample until the pink coloration of the sample will be darker or the same than the progesterone control A provides with the kit. During the breeding season, if the sample of the mare is darker than the progesterone control A, then the mare is in estrus.

To Confirm Estrus or non-Estrus: A single sample should be taken on the day of suspected estrus. Breeding is indicated when the progesterone is below 3ng/mL (the tested sample is darker than the progesterone control A provides with the kit), and the mare is showing behavior corresponding to being in heat.

To Detect Silent Estrus: 6 days prior to the expected heat cycle, collect and test samples every other day until the progesterone falls below 3ng/mL (the pink color of the tested sample is darker than the progesterone control A provides with the kit).

Collection of the sample:

Plasma:

Collect a heparinised blood sample in a heparin-coated tube and separate the plasma from the red blood cells by centrifugation. Use the plasma for the measurement. EDTA-plasma is not recommended.

Serum:

Collect a sample of blood in the usual manner in a dry tube; 1 mL of blood is enough. Allow to coagulate at room temperature. Use the serum on the top for the measurement.

Note: It is not recommended to use serum in SST tube because progesterone value can be falsely decreased.

F. PRECAUTIONS

- Keep the kit refrigerated (2-6°C). DO NOT FREEZE.
- Do not use wells more than once. Wells are coated with anti-progesterone.
- The solution in bottles A and B contain preservative.
- When one empties the contents of the wells into the sink, rinse away with a large amount of tap water.
- For *in vitro* veterinary diagnostic use only.
- Keep out of reach of children.
- Do not pipette solutions by mouth.
- If the product splashes into the eyes or onto the skin, wash thoroughly with tap water.
- For more information, contact technical services at Biovet Inc.

G. KIT CONTENTS

Quantities	Reagent and Materials
1 x 1,0 mL	Ready-to-use low standard A (3ng/mL/mL)
1 x 1,0 mL	Ready-to-use high standard B (10ng/mL)
1 x 8,5 mL	Ready-to-use Conjugate
1 x 8,5 mL	Substrate Buffer
1 tablet	Substrate
4 x 8 wells	Coated Microwells
35	Plastic Pipettes
1	Rubber Bulb for Plastic Pipettes

References: 1. Pinto CRF, Kozink DM, Whitacre MD, Whisnaut CS. Utilization of a semi-quantitative ELISA progesterone kit in broodmare management. J Ani Repro Sci 94 (2006) 204-206

OVUCHECK® PREMATE EQUINE

INSERT

Kit for Determination of the Level of Serum or Plasma Progesterone in Mare¹.

Cat. No.: E001

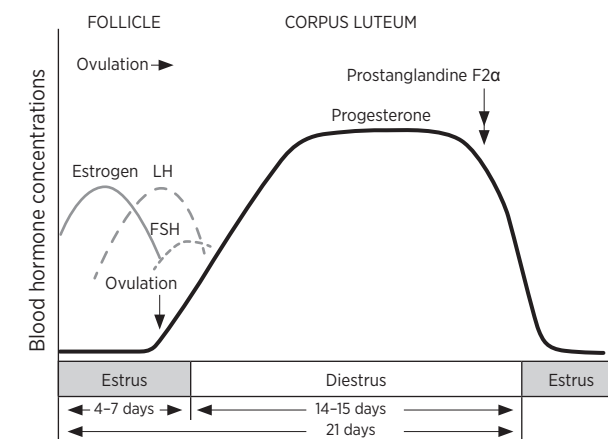
A. INTRODUCTION

OVUCHECK® PREMATE is a semi-quantitative test for the measurement of progesterone in a drop of plasma or serum. The quantity of progesterone present is indicated by a change in color, which is compared with progesterone standards (low, intermediate and high).

This kit is quick and simple to use. It provides useful information:

- when the mare's genital exam is contradictory;
- when the mares do not show regular estrous cycle during transition period or during the reproduction season;
- to confirm ovulation;
- to follow the first trimester of high risk gestations;
- to maximize the gestation rate during embryo transfer programs;
- for reproduction of small or miniature mares.

B. PHYSIOLOGICAL BASIS



The mare is a seasonal polyestrous animal. During the breeding season, the non-pregnant mare will have many estrous cycles. The estrous cycle is divided in ovulatory period (estrus or follicular phase) and in interovulatory period (diestrus or luteal phase). During the breeding season, the length of estrus cycle is 19-24 days (averaging 21.5 days).

Estrus: Estrus occurs when the mare is sexually receptive to the male. The progesterone concentration is low (< 1 ng/mL). The length of estrus is 4 to 7 days and ovulation occurs 24 to 48 hours before the end of estrus. The best time for breeding is just prior to the ovulation or at the end of estrus (D4-D5). A few mares may not show regular estrous behavior or have uterine edema detected by transrectal ultrasonography.




D. OPERATING PROCEDURE

Bring all the components to room temperature before use (about 30 minutes).

Make sure every solution is properly mixed before use.

Preparation of the substrate:

- 
- Carefully remove the screw cap from bottle D. Then, take out and discard the stopper.
 - Without touching it, eject the substrate tablet into bottle D by pressing the back of the metal foil.
 - Place the supplied nozzle into bottle D. Put on the cap and screw firmly to ensure the nozzle fits securely into the neck of the bottle.
 - Mix at regular intervals, until the tablet has completely dissolved (15 to 30 minutes). The solution must now be yellow.

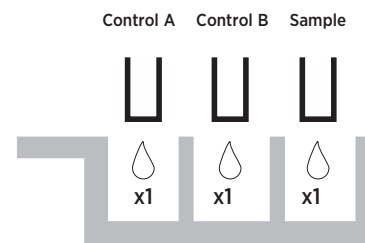
Storage of the substrate:

- Write the date on the label. The mixture prepared in bottle D is now stable for up to three months if kept refrigerated (2–6°C).
- This solution can be frozen for a longer period of conservation. The expiration date is then shown on the outside label on the kit box. You can aliquot solution D, to avoid repeated freeze/thaw cycles. For example, you can use insulin syringes (0.6 mL per syringe). This way, you will only have to take one syringe out of the freezer each time you perform the test.
- The colour of the solution of bottle D (when activated) can change with time but this does not affect results interpretation.

Carrying out the test:

Two standards are provided (A and B). They must both be used each time an assay is performed to validate the test.

- Take out a strip of wells from the plastic bag.
- Select the necessary number of wells (2 + n, n being the number of samples to test) by breaking the plastic between the wells. Put the unused wells back in the plastic bag. Mark for identification purposes the top of the first well you will use.



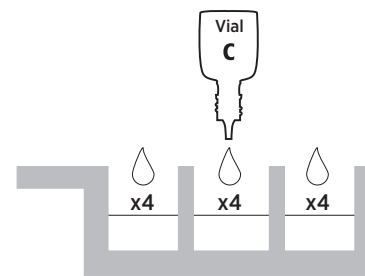
Run up to ten tests at a time individually, or 30 tests in one batch.

Always use a new pipette for each sample or standard.

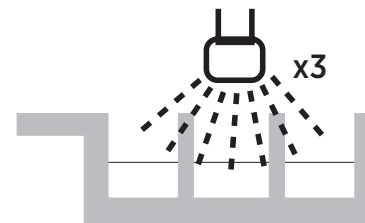
Keeping the pipette vertical, add:

- 1 drop of low standard (A) to the first well.
- 1 drop of high standard (B) to the second well.
- 1 drop of the sample to be tested to the third well. Each sample to be tested shall be added to a different well. Wells must either contain a standard or a sample, not both.

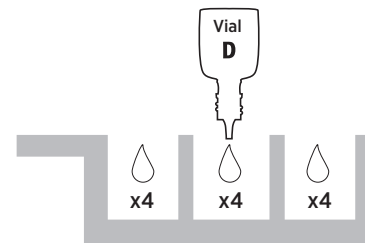
Important note: If the pipette is not held in a vertical position, the drop volume could be smaller and affect the accuracy of the test.



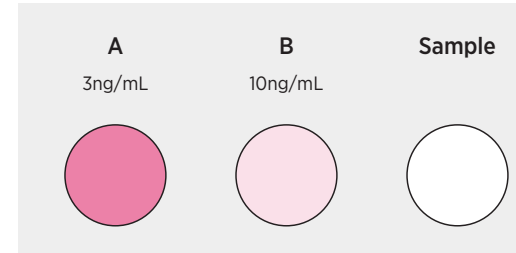
- Keeping bottle C vertical, add 4 drops of reagent C to each well.
- Cover the wells and incubate for 15 minutes at room temperature.



- Empty the contents of the wells into the sink and gently rinse the wells three times, using lukewarm tap water. Dry by tapping onto absorbent paper. To allow better colour differentiation, avoid overdrying.



- Keeping bottle D (or insulin syringe) vertical, add 4 drops of the prepared substrate to each well.
- Cover the wells to protect them from light and incubate for 15 minutes at room temperature.**



Agitate the wells gently to mix contents and compare the color of the sample to the colors of standards low (A), and high (B).

E. INTERPRETATION OF THE RESULT

To help visualize the results, place the wells on top of a white background and look at the wells from the top.

First check the 2 standards, low and high. The low standard A (3ng/mL) should be the darkest (dark pink), the high standard B (10ng/mL) should be the lightest. The intensity of the coloration is inversely proportional to the sample's concentration of progesterone.

If the sample is as pink as or darker than the low standard...



If the sample is as pink as or darker than the low standard A (3ng/mL), the progesterone concentration is equal or lower than 3ng/mL.

There is the absence of a functional corpus luteum (CL3).

During winter months, this result suggests that the mare is in anestrus. During the breeding season, this result suggests that the mare is in the estrus phase and if the follicles are > 35 mm the mare may be introduced to the stallion.

If the sample color is in-between the low A and the high B standard...



If the sample color is lighter pink than A but darker than B (in-between the low A (3ng/mL) and the high standard B (10ng/mL), the progesterone concentration is between 3 and 10ng/mL.

The mare is in diestrus (in post-ovulation or at the end of diestrus).

Test again in 2-3 days.

If the sample color is lighter than the high standard...



If the sample color is lighter than the high standard B (10ng/mL), the progesterone concentration is greater than 10ng/mL.

The mare is in diestrus.

Test again in 2-3 days.