The Power Of Progesterone

Tracking the key hormone from estrus through pregnancy

"To understand where we are, we have to know where we have been" — Old Kentucky Mountaineer

By R.H. Douglas, PhD

This advertisement, from the June 5, 1875, Kentucky Gazette, offers a clear illustration of what breeding horses was like 118 years ago in the Lexington, Kentucky area:

"The following celebrated trotting stallions will make the season of 1875 at Fairlawn Stock Farm, adjoining the city of Lexington, Ky.:

"ALMONT — the great sire of trotters. Almont will be limited strictly to sixty mares, besides his owners, at $150 the season.

"ADMINISTRATOR — will be limited to 75 approved mares at $100 the season."

And on the Thoroughbred side:

"ASHLAND PARK — Stallions: GEORGE WILKES, $100 the season and HONEST ALLEN, $75 the season. Terms: Money due at the time of service, privilege of breeding back should the mare not prove with foal. Season commences March 1st and ends July 1st, 1875. Mares kept at reasonable rates. All accidents and escapees at owners risk. David Muckle, Superintendent.

"Persons wishing to breed are invited to call and see these two horses before making other arrangements. Mares shipped on cars (railroad) will be met by Wilson and Bros at Lexington and kept in their livery stable until sent for by me. I will send a careful groom every Wednesday and Saturday to Lexington and any mare left at Wilson and Bros stable will be taken out free of charge and returned when done with the horse. Good grass furnished at 10 cents per day or grain at 25 cents per feed.

"All care will be taken but will not be responsible for accidents or escapes.

"All bills for grazing, feed and for the groom must be paid before removal of mares — inquires to T. B. Muir."

Several interesting facts can be learned about the horse breeding industry 100 years ago from reading these ads:

1. It appears many mares escaped while being boarded at breeders’ farms.

2. Even before artificial insemination was developed and routinely used by Standardbred breeders, books of 60 to 80 mares were commonly bred by natural cover.

3. Keeping mares for breeding was much cheaper then than now. However, it was about this time that an axiom was established about breeding horses: "If you want to lose a friend, give him a broodmare. If you want to bankrupt him, give him two."

4. In order to get a large number of mares to a stallion, constant advertising was required.

5. This newspaper and other information suggests that the live foal rate during this era was about 70%. Current data for 1991 published by The Jockey Club indicates the live foal rate for 1991 in North America was about 60%.

6. Neither Mr. Muckle nor Mr. Muir had ever heard of hormones, including progesterone, since it had not been discovered.

We've come a long way since 1875. Or have we? Is your live foal rate greater than 70%? That is, for every 10 mares you breed, do you get seven live foals? If not, maybe some of the information in this article will help you be more efficient than the breeders in central Kentucky during 1875.

Have we really improved? According to the Thoroughbred annual report of Live Foals by Stallion, 7,795 stallions covered 76,652 mares in 1990, resulting in 46,296 foals in 1991. This is equivalent to a live foal percentage of 60.4% for all of North America. Among the different states breeding over 1,000 mares, Kentucky had the highest live foal rate at 69.4%. There are many well-managed farms which have a live foal rate greater than 80%.

This is remarkable in view of the restraints placed on Thoroughbred breeders. No artificial insemination is allowed, and often only one cover or breeding per estrous cycle is possible due to the large number of mares bred to some stallions. For example, in Kentucky in 1990, 12 Thoroughbred stallions bred more than 60 mares by natural cover, three stallions bred...
106, 107 and 116 mares, respectively, and still had a live foal rate of over 70%. To accomplish this between February 10 and July 15 — a period of 155 days — some stallions are bred three times per day. In 1875, most mares were bred every other day until they went out of heat. At this time of year, we often must make an appointment (booking) to breed Thoroughbred mares two, three, and sometimes four days in advance. As a result, a great deal of data is required to accurately make these bookings.

Many recent advances have been made which have greatly improved our conception and live foal rates, even in the face of the difficulties of breeding mares by natural cover. One of the major problems, and often one of the most expensive to manage, is the spread of venereal disease when large numbers of mares are bred to a given stallion by natural cover. This problem is significantly reduced in breeds using AI, which eliminates the need for genital contact between mare and stallion.

Most of the venereal infections in mares and stallions involve bacterial organisms and occasionally fungi. New treatments involving uterine lavages and potent antibiotics, some of which are given systemically and some of which are given intratuterinely, which are sometimes used in combination with hormones such as oxytocin and prostaglandin F
alpha, are now required to successfully treat some of the more difficult cases of uterine infections (endometritis).

Advances In Hormonal Control

Another area in which great advances have been made is in understanding hormonal control — the endocrinology of reproductive function in mares and stallions. Many applications are being made using exogenous hormones to control or improve reproductive performance. Our database on endogenous hormonal levels and their dysfunction is also expanding and is rapidly being assimilated in the veterinarian’s and farm manager’s decision-making processes.

Progestins, which include both synthetics such as Regu-mate® and native progesterone and its metabolites, have received much attention, both in terms of controlling ovarian function and in maintaining pregnancy in mares at risk of losing pregnancies. In addition, use of progestrone assays is now commonplace for most veterinarians and managers. This article will give you an overview of progestrone assays and values in sera and what they mean to your management decisions.

Farm Manager’s Guide To Progesterone Assays

1. Establish ovulatory status of all mares
   - If progesterone level is greater than 1.0 ng/ml, mare is ovulatory
   - If progesterone level is less than 1.0 ng/ml, mare is anovulatory or should be in heat
   - Routinely sample mares when they arrive at your farm
   - Check foaling mares in which foal-heat estrus was not observed
   - Check any mares not showing estrous behavior at normal intervals

2. Identify mares which have early failure of the primary corpus luteum
   - Sample at day 12 post-ovulation. Values should be greater than 4.0 ng/ml
   - Mares with values less than 2.0 ng/ml may have luteal insufficiency, which is most likely due to uterine infection. If mare is not pregnant when ultrasounded, reculture reproductive tract. Uterine lavage may be indicated.
   - Mares with values greater than 10.0 ng/ml likely double ovulated and should be checked carefully for twins

3. Reinforce teasing program
   - Determine status of mares missed by the teaser
   - Reduces amount of teasing necessary for good management

4. Determine which mares may need progestin therapy to support pregnancy
   - Measure progesterone levels at first ultrasound or manual pregnancy check
   - Use progestin therapy in mares testing below normal for that stage of pregnancy. Expect 8 to 10% to test below normal
   - Retest at various intervals of pregnancy to determine if mares that were low in progesterone have now become normal and can be taken off progestin therapy

5. Identify low progesterone levels during pregnancy
   - Test at various stages of pregnancy. When levels are below normal, retest to confirm and place on progestin therapy
   - Don’t ship mares with low serum levels of progesterone. If possible, wait until at least 100 days of pregnancy before shipping. If progesterone levels are still low, place mare on progestin therapy prior to shipping
   - If progesterone levels are low prior to weaning, place on progestin therapy before weaning
   - If mare colics or is otherwise seriously stressed, measure progesterone

Progesterone is a steroid hormone that can be produced only by a few endocrine tissues, including the corpus luteum (Plate 1), the conceptus (Plate 2) and the adrenal gland. No appreciable amounts of progesterone are present in the mare’s blood sera during estrus (heat). Thus, blood levels will be less than 1.0 nanograms per milliliter (ng/ml) during this time. A
nanogram is one billionth of a gram.

Obviously, very sensitive and accurate assay methods are required to reliably measure progesterone concentrations in sera. The slightest error in handling the volume of sera to be assayed can significantly alter the results reported and affect the interpretation of what the mare is doing hormonally. Precision is improved when all samples are assayed in duplicate and the variation between duplicates calculated. Variations greater than 10% reflect pipetting error or some other errors within the assay, and the sample should be reassayed.

Immediately after the ovulatory ovarian follicle releases the oocyte (ovulation) near the end of estrus, cells within the follicle begin to enlarge and become luteinized, forming a structure within the collapsed ovulatory follicle called the yellow body (corpus luteum). Although in mares the body is more red than yellow, this structure is commonly called the corpus luteum in all mammalian species.

By day two after ovulation, sera levels of progesterone in the mare should exceed 1.0 ng/ml. Table 1 illustrates how sera levels of progesterone change throughout the pregnancy. When mares are not pregnant, the uterus releases prostaglandin F_2alpha into the peripheral circulation and kills or lyses the corpus luteum.

| Table 1. Normal Serum Levels Of Progesterone As Determined By Radioimmunoassay |
|-----------------------------|------------------|----------------|
| Days after ovulation        | Ng/ml            |                |
| 0                          | Less than 1      |                |
| 2                          | 1.0 to 3.0       |                |
| 5-12                       | 4.0 to 10.0      |                |
| 15-18                      | 3.0 to 10.0      |                |
| 21                         | 3.0 to 10.0      |                |
| 25-30                      | 2.5 to 10.0      |                |
| 45                         | 4.0 to 10.0      |                |
| 60                         | 4.0 to 15.0      |                |
| 80 to 315                  | 5.0 to 10.0      |                |
| 316 to term                | 20.0 to 40.0+    |                |

Results are those used by BET Laboratories and were generated by a radioimmunoassay (RIA) which measures progesterone and some of its major metabolites.

Plate 1. A newly formed corpus luteum on the ovary at the site of what was the ovulatory follicle. This structure is the source of progesterone for approximately the first 110 days of pregnancy.

When sera levels fall below 1.0 ng/ml again, the mare returns to estrus. As long as progesterone remains above 1.0 ng/ml, she will not show estrous behavior. The primary corpus luteum remains present through about 120 days of pregnancy and is the major source of progesterone during this period of pregnancy.

To demonstrate the importance of this structure to pregnancy maintenance one need only consider the following data: When the ovaries containing the corpus luteum are removed from pregnant mares during the first 100 days of pregnancy, 100% will abort the pregnancy. When the ovariec- tomy is performed in the same stage of pregnancy and the mares are given progesterone therapy, none of the mares aborted when the appropriate level of progesterone was given. When ovariectomy is performed after 120 days of pregnancy — when the feto-placental unit has become the major source of progesterone — none of the mares aborted.

What causes the corpus luteum to be maintained and thus allow the pregnancy to survive? Plate 2 shows what a 14-day-old equine conceptus looks like. It is spherical and has been rolling around throughout the interior lumen of the uterus up until this time. By some unknown mechanism the conceptus prevents the uterus from releasing prostaglandin F_2alpha, and the corpus luteum survives if all the messages are sent properly and in a timely fashion.

Another way of demonstrating this utero-ovarian relationship is to remove the uterus from the mare about seven days after ovulation. When this is done, the corpus luteum which normally only survives for about 14 days will now be present for about 100 days. The important point is that there is a symbiotic relationship between the corpus luteum and the conceptus. One can not endure without the other.

Hormones are often secreted at different rates at different times during
nancy loss in mares older than 14 years.

We have focused much of our research in the last few years on the older mare. We know she is more likely to have chronic endometrosis, which is the biggest problem one must manage. But she is also more likely to produce lower than normal amounts of both progesterone and another hormone important to reproductive performance, thyroxine \( T_4 \). When appropriate antibiotic therapy combined with adequate hormonal assays and hormonal therapy are used in the management of these older subfertile mares, pregnancy loss can be reduced to the level of that seen in younger mares.

This is not an inexpensive endeavor and can not be done casually. One must know as much as possible about these mares on an individual basis and religiously administer the indicated hormonal therapy and monitor blood levels of various hormones to ensure they are within the normal range. For some genetically valuable mares, this intense effort may be worth it.

Of course, we could reduce all this management by simply performing an embryo transfer from the old, subfertile mare into a young, fertile recipient. Some breeds, like the Thoroughbred, however, do not permit embryo transfer so we must do our best with mother nature and the aging process. When successful, the results are very rewarding.

A summary of the uses of progesterone assays is given in the Farm Manager's Guide on page 15. When done properly, use of hormonal assays will greatly improve your management efficiency and save money for both you and your client, since you will have more mares in foal in a shorter number of days and you will have more live foals on the ground each year. Even in the years you are not successful with a given mare, you may be able to explain accurately to your client why she failed to produce a live foal and map out different management protocols for next season.

Author R.H. Douglas, PhD, is president of BET Laboratories in Lexington, Kentucky. He pioneered the use of radioimmunoassay (RIA) in the mare and has developed complete endocrine profiles for the mare, stallion, foal and performance horse. He is currently working on microencapsulated delivery systems for progesterone and other hormones. Douglas is an MHB Contributing Editor.