WHAT’S NEW IN EQUINE THERIOGENOLOGY-
A review of current topics for practitioners

Amanda C. Ragon, DVM, Diplomate ACT
Equine Fertility Specialties, PLLC
Williamston, MI 48895

MARE:

Control of the Estrous Cycle: Estrus Suppression, Estrus Induction, Ovulation Induction

Controlling the estrous cycle in the mare continues to be essential for mare owners and veterinarians. Suppressing estrus behavior in performance mares and managing follicular development and ovulation in broodmares are significant undertakings that we strive to improve upon. Research in these areas has provided new information that can be used in daily practice.

How to suppress estrus is a common request from horse owners. Daily altrenogest therapy, while still considered the “gold standard” of estrus suppression, is costly, time-consuming and requires special handling by those who are administering this medication to mares. Finding alternatives to daily altrenogest therapy is the current focus of many researchers.

Compounded altrenogest preparations

Altrenogest compounded in sustained release preparations is an effective way of suppressing estrus in mares. Long-acting injectable formulations of 225 mg altrenogest last approximately 12 days. Altrenogest (500 mg) suspended in microparticles is reported to last up to 30 days in many mares.

Oxytocin

Oxytocin administration during diestrus appears to block luteolysis and extend CL function. Injection of 60 I.U. of oxytocin once daily intramuscularly on days 7-14 maintained CL function (progesterone levels >1ng/ml) for 50 days in over 60% of study mares. A similar study in New Zealand found that lower doses of oxytocin (10 I.U.) did not produce the same results. Most mares were reported to tolerate the daily injections well and side effects were minimal; however, it is important to determine the day of ovulation in order for this protocol to be successful.

Plant Oils

Intrauterine infusion of 1ml of fractionated coconut oil or peanut oil on day 10 induced prolonged CL function in 92% of mares in treated groups. In contrast, infusion of mineral oil did not produce similar results. Originally, 10 mg of estradiol was added to the plant oil infusion, but the study revealed that estradiol was not needed to achieve prolonged CL function. A more
recent study investigated the dosage of fractionated coconut oil necessary to prolong luteal function, but found that both doses lowered progesterone levels during diestrus and failed to prolong luteal function.

**Intrauterine marbles**

Glass or plastic balls steriley placed into the uterus immediately after ovulation have been used to extend CL function and suppress estrus since described in 2003. Unfortunately, time and experience have shown this technique to be less effective at suppressing estrus and more likely to result in complications (endometritis, pyometra, glass shards, marbles remaining during pregnancy) than other available methods of estrus control.

**Control of Estrous Cycle-Induction of Estrus**

**Prostaglandins**

The use of injectable forms of prostaglandin F2α (PGF) has been an invaluable addition to breeding management in both horses and cattle. The standard practice of administering PGF to mares 5-6 days after ovulation to cause luteolysis and return the mare to estrus is nothing new; however, recent studies are challenging our long held belief that the CL is not responsive to PGF before it is at least 5 days old. New research has described an “antiluteogenic” effect of PGF when used as early as 24 hours after ovulation: in other words, the early CL is responsive to luteolysis before it is fully functional. Mares treated twice daily with 10mg of dinoprost on days 0, 1, 2 and once daily on days 3 & 4 failed to show a rise in progesterone greater than 1 ng/ml. Interovulatory intervals were significantly reduced compared to control mares (average of approximately 12 days compared to 21.) Pregnancy rates when compared to control mares were equivalent. Utilizing the antiluteogenic effect of PGF may represent a new approach to manipulate the mare’s cycle.

**Control of Estrous Cycle-Induction of Ovulation**

Human chorionic gonadotropin (hCG) has long been used to induce ovulation in broodmares. The GnRH analogues, deslorelin, histrelin and buserelin, have become increasingly more popular and touted to be more effective at ensuring ovulation within a predictable time span than hCG. Deslorelin is commercially available and FDA approved, while other GnRH analogues are available as compounded products. While multiple studies have shown that the GnRH analogues deslorelin and histrelin are equally effective at inducing ovulation within 2 days of treatment as hCG, no study has determined that these substances are significantly more reliable than hCG. Buserelin is less potent than either deslorelin or histrelin. In terms of choosing a product on the basis of FDA approval, it is worth remembering that hCG is not approved for use in the horse although it has been used commonly since 1939.
Endometritis

Biofilms

Biofilms are an extracellular matrix produced by bacteria (particularly *E. coli* and *Pseudomonas aeruginosa*, but also *Strept.zooepidemicus*) which coat the endometrium and protect the bacterial population from both antibiotics and the host’s immune system, leading to a population of resistant and persistent organisms. Biofilms cannot be diagnosed through standard culture, cytology or biopsy, but should be suspected in cases of recurrent infections that don’t clear when treated with appropriate antibiotic therapy.

Disruption of the biofilm to allow antibiotic access to the bacterial community has been tried with several treatment options: the mucolytics N-Acetylcysteine and hydrogen peroxide, and a buffered chelating agent, tris-EDTA (Tricide® solution.) Typically, treatment involves intrauterine infusion with one of the suggested treatment solutions, allowing the solution to sit overnight in the uterus and uterine lavage the following day. Uterine lavage is followed by the infusion of appropriate antibiotics +/- or systemic antibiotic therapy. Several treatment cycles are likely to be necessary. Success rates of such treatments differ greatly between *in vitro* and *in vivo* models.10

Immune modulators

Treatments that modulate the immune response and their role in the treatment of endometritis have been commonly used with varying degrees of success reported in the literature. Glucocorticoids (prednisolone and dexamethasone) and NSAIDs have been reported to help control post-breeding endometritis. Immunostimulants such as *Mycobacterium phlei* cell wall extract (MCWE) and *Propionibacterium acnes* are purported to increase the host immune response, but studies incorporating these treatments often disagree about their effectiveness.11

Newer therapies include platelet-rich plasma (PRP) which is reported to improve pregnancy rates and decrease post-breeding uterine fluid in a group of susceptible mares12 and “promoting factors” which induce active growth of dormant/inactive bacteria (specifically *Strept.zooepidemicus*) in order to make this population of bacteria susceptible to intrauterine and systemic antibiotic therapy.13

Retained fetal membranes

A technique (first described in 1917) has been “reinvented” for the removal of retained fetal membranes. Termed the “Zeddam technique” from the group of Dutch veterinarians who have promoted it with a YouTube video, it involves the catheterization of an umbilical vessel with either a small nasogastric tube or stallion catheter, when is then attached to a garden hose. Tap water is then infused into the vessel while gentle traction in applied to the umbilical cord.14
Testing for Reproductive Disease

**AMH**

Blood levels of anti-Müllerian hormone (AMH) are very useful in detecting the presence of granulosa-thecal cell (GCT) tumors in the mare. Serum concentrations of AMH in mares with GCT are much higher than either pregnant or cycling mares, and decrease after tumor removal. There is no effect of cycle stage on month of gestation on AMH concentrations in serum. In addition, AMH levels may be useful in predicting follicular reserve in aged mares.

**SAA**

Serum amyloid A (SAA) is an inflammatory acute phase protein that has shown promise as a marker for placentitis in women. SAA levels also increase in mares with experimentally induced placentitis, but clinical signs preceded the rise in SAA levels. Another study compared SAA levels to WBC and fibrinogen in experimentally induced cases of placentitis. Neither WBC nor fibrinogen levels were helpful markers in identifying placentitis. Increases in SAA appear to be a consistent finding of placentitis, but it remains to be seen if this will prove to be an easy method of early disease detection.

**STALLION:**

**Aids in Semen Processing**

Semen quality can be improved and the number of spermatozoa preserved in the subfertile stallion with a number of relatively simple techniques: standard centrifugation, centrifugation using a cushion fluid, and single-layer centrifugation. Stallions whose semen quality diminishes over time may benefit from the removal of seminal plasma prior to resuspension in an appropriate semen extender. Cushioning fluids are helpful in protecting spermatozoa from the deleterious effects of centrifugation and also in maximizing the sperm harvest. Single layer centrifugation uses a silica-particle solution to remove dead, immotile, immature and damaged cells and bacteria from the ejaculate prior to breeding.

In addition, the availability of commercially prepared semen extenders has greatly expanded in the last few years. Many formulas specifically provide support for stallions whose semen loses viability during cooling and shipping.

**Nutritional Support for the Subfertile Stallion**

The use of “nutraceuticals” to increase fertility in the stallion is an appealing concept for stallion owners. Subfertile stallions show more improvement in semen parameters (especially during cooled storage) than normal stallions. The main focus of nutritional intervention is changing the ratio of DHA (and omega 3 fatty acid) to DPA (an omega 6 fatty acid) in the diet. The typical equine diet favors DPA over DHA, due to the corn and soybean oil content. Flaxseed oil (rich in...
alpha-linolenic acid) is a commonly used supplement, and is a precursor to DHA, but the conversion to DHA is an inefficient process in the horse. Supplements which include DHA precursors (such as flaxseed oil) plus preformed DHA and antioxidants have been shown to increase fertility in stallions. 20

REFERENCES: