How Can You Be Sure That Your Mare No Longer Needs Progesterone Supplementation?

Pregnancy is maintained by progesterone secreted by the corpus luteum of the ovary in the first few months (Conley 2016). For decades, inadequate luteal progesterone secretion (nominally less 4ng/ml in the mare's blood) has been taken as a cause for pregnancy loss and a rationale for supplementation. Clinically, this is achieved with administration of altenogest (commonly Regu-Mate© or altrenogest compounded otherwise) or alternative preparations believed to have progesterone-like activity.

Is supplementation necessary for the entire length of equine gestation?

The initial hormonal support of pregnancy in mares switches from progesterone secretion by the ovary (corpus luteum and secondary luteal structures) to the synthesis and secretion by the placenta of an active metabolite of progesterone, called 5α-dihydroprogesterone (DHP), after about the 4th month of pregnancy (Scholtz, et al. 2014). DHP is actually as potent and as effective as progesterone, and is fully capable of maintaining pregnancy on its own. Progesterone slowly disappears from the circulation and is essentially undetectable in the second half of pregnancy (Conley 2016; Scholtz et al. 2014). There has never been a case reported in the literature implicating the placenta of providing insufficient hormonal support as a cause of pregnancy failure. Therefore, when the placenta takes over the hormonal support of pregnancy, there is no clinical rationale to continue supplemental hormonal support of pregnancy in mares.

How can you be sure the placenta has taken over from the ovary (corpus luteum) in supporting pregnancy in mares?

It is possible to confirm that the placenta has taken over from the ovary when the concentration of DHP (secreted by the placenta) exceeds that of progesterone (secreted by the ovary) in blood and, once confirmed, is evidence that supplementation is no longer necessary (Conley 2016).

How can I measure DHP in my mare?

Until now, there has been no way for practitioners, managers or owners to assess hormonal support of pregnancy by the placenta because there has never been an assay method that could measure DHP and able to distinguish progesterone from DHP. Almost all commercially-available assays for progesterone are **immuno**-assays meaning that they use antibodies to detect the hormone. The antibodies used in progesterone assays can rarely distinguish DHP from progesterone, meaning that there is virtually no way to measure both progesterone and DHP by immuno-assay (Wynn, et al. 2018). No immuno-assays for DHP itself exist. Accurate and specific measurement of progesterone and DHP in serum or plasma samples can be done but currently only by a technique that employs mass spectrometry (Legacki, et al. 2016; Scholtz et al. 2014). It requires extremely expensive, specialized equipment which has only previously been accessible to researchers. This laboratory has utilized mass spectrometry to measure progesterone and DHP for research for many years, and now has been given access to an instrument that can be used for clinical samples on a weekly basis. In short, we are now offering the determination of progesterone and DHP quantitation in serum or plasma from pregnant mares using mass spectrometry to verify whether or not the placenta has taken over the support of pregnancy and it is considered safe to take mares off progesterone supplementation.

Why should I consider taking my mare off supplementation?

Breeders can improve the efficiency of their programs by monitoring progesterone and DHP in their mares. Providing supplementary hormonal support for pregnancy involves costs to purchase and effort to administer the supplements. To be sure, the active ingredient in many of these preparations is the synthetic steroid altrenogest which is a very potent substitute for progesterone. But altrenogest also has potent androgenic (masculinizing, testosterone-like) activity (McRobb, et al. 2008) and it is identified as a potential reproductive toxicant (Sigma-Aldrich, Material Safety Data Sheet, Product #33994). Product information provided by the manufacturer of Regu-Mate© states that the product can cause "masculinization of the female genitalia" in rats. The package insert recommends "extreme caution" when using it, especially so for pregnant women.

From Regu-Mate©, product insert, MERCK Animal Health:

> *HUMAN WARNINGS: Skin contact must be avoided as Regu-Mate® (altrenogest) Solution 0.22% is readily absorbed through unbroken skin. Protective gloves must be worn by all persons handling this product. Pregnant women or women who suspect they are pregnant should not handle Regu-Mate® (altrenogest) Solution 0.22%. Women of child bearing age should exercise extreme caution when handling this product. Accidental absorption could lead to a disruption of the menstrual cycle or prolongation of pregnancy. Direct contact with the skin should therefore be avoided. Accidental spillage on the skin should be washed off immediately with soap and water.*
> *INFORMATION FOR HANDLERS:*
> ***WARNING**: Regu-Mate® (altrenogest) Solution 0.22% is readily absorbed by the skin. Skin contact must be avoided; protective gloves must be worn when handling this product.*

Consistent with results in rats, studies in pregnant mares have shown that fillies foaled from mares treated with Regumate© throughout pregnancy were born with clitoromegaly, a symptom of androgen exposure, which persisted into their first breeding season at least. Although all of these fillies achieved pregnancy in their first breeding season (Naden, et al. 1990), the long term consequences on fertility as these mares age is unknown. However, studies in other species indicate that androgen exposure *in utero* can disrupt normal ovarian development and function later in life (Abbott, et al. 2006; Filippou and Homburg 2017; Padmanabhan and Veiga-Lopez 2011), with a consequent loss of fertility. While there is no direct evidence that products with androgenic activity can cause a decrease in fertility in filly foals in their first breeding season (Naden et al. 1990), *our recommendation for best safe practices is to limit, as much as possible, the exposure of female fetuses to androgens during pregnancy.*

Is there any guarantee, if DHP is higher than progesterone in blood, and I take my mare off progesterone supplementation, that there is no risk of abortion or pregnancy loss at some later date?

Pregnancy can be lost at any point in gestation and for a multitude of reasons, infectious agents among other causes. No one can say that progesterone supplementation is absolutely necessary to maintain a pregnancy in mares with low progesterone (Allen 2001). No one can say that even after the placenta takes over support of pregnancy that abortion cannot occur later. There are also some circumstances,

such as in cases of placentitis, in which supplementation may be indicated and re-introduced (LeBlanc 2010). The known effects of altrenogest on reproductive development of filly foals and the danger to those administering it, especially pregnant women, must be balanced against identified risks to fetal development and potential future fertility. We believe the safest practice is to cease supplemental support of equine pregnancy when DHP is expected to exceed progesterone in the mare's blood at about 110-120 days of gestation (Scholtz et al. 2014). This can now be confirmed and verified by analysis of blood levels using mass spectrometry.

The Clinical Endocrinology Laboratory is adding this clinical test as available to referring veterinarians and all clients. Sample submission is as for other offered tests (serum or plasma, 2 mls). Feedback on the final outcome of pregnancies is invited and will be much appreciated so that we can collect, monitor and evaluate data retrospectively season by season.

Supporting scientific literature:

Abbott DH, Padmanabhan V & Dumesic DA 2006 Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. *Reprod. Biol. Endocrinol* **4** 17.
Allen WR 2001 Luteal deficiency and embryo mortality in the mare. *Reprod. Domest. Anim* **36** 121-131.
Conley AJ 2016 Review of the reproductive endocrinology of the pregnant and parturient mare. *Theriogenology* **86** 355-365.
Filippou P & Homburg R 2017 Is foetal hyperexposure to androgens a cause of PCOS? *Hum Reprod Update* **23** 421-432.
LeBlanc MM 2010 Ascending placentitis in the mare: an update. *Reprod. Domest. Anim* **45 Suppl 2** 28-34.
Legacki EL, Scholtz EL, Ball BA, Stanley SD, Berger T & Conley AJ 2016 The dynamic steroid landscape of equine pregnancy mapped by mass spectrometry. *Reproduction* **151** 421-430.
McRobb L, Handelsman DJ, Kazlauskas R, Wilkinson S, McLeod MD & Heather AK 2008 Structure-activity relationships of synthetic progestins in a yeast-based in vitro androgen bioassay. *J. Steroid Biochem. Mol. Biol* **110** 39-47.
Naden J, Squires EL & Nett TM 1990 Effect of maternal treatment with altrenogest on age at puberty, hormone concentrations, pituitary response to exogenous GnRH, oestrous cycle characteristics and fertility of fillies. *J. Reprod. Fertil* **88** 185-195.
Padmanabhan V & Veiga-Lopez A 2011 Developmental origin of reproductive and metabolic dysfunctions: androgenic versus estrogenic reprogramming. *Semin. Reprod. Med* **29** 173-186.
Scholtz EL, Krishnan S, Ball BA, Corbin CJ, Moeller BC, Stanley SD, McDowell KJ, Hughes AL, McDonnell DP & Conley AJ 2014 Pregnancy without progesterone in horses defines a second endogenous biopotent progesterone receptor agonist, 5alpha-dihydroprogesterone. *Proc. Natl. Acad. Sci. U. S. A* **111** 3365-3370.
Wynn MAA, Esteller-Vico A, Legacki EL, Conley AJ, Loux SC, Stanley SD, Curry TE, Jr., Squires EL, Troedsson MH & Ball BA 2018 A comparison of progesterone assays for determination of peripheral pregnane concentrations in the late pregnant mare. *Theriogenology* **106** 127-133.