



Improving the formation and function of the corpus luteum in the mare

Melhorando a formação e função do corpo lúteo na égua

Harald Sieme^{1,5}, Johannes Lüttgenau², Jutta Sielhorst¹, Gunilla Martinsson³, Heinrich Bollwein²,
Selina Thomas⁴, Dominik Burger⁴

¹Clinic for Horses, Unit for Reproductive Medicine, University of Veterinary Medicine Hannover, Hannover, Germany.

²Clinic of Reproductive Medicine, Vetsuisse-Faculty University Zürich, Zürich, Switzerland.

³National Stud Celle, Celle, Germany.

⁴Swiss Institute of Equine Medicine, Agroscope and University of Berne, Avenches, Switzerland.

⁵Correspondence: harald.sieme@tiho-hannover.de

Abstract

The corpus luteum (CL) is a pivotal structure in the regulation of the estrous cycle and in the establishment of pregnancy. It is one of the most vascularised organs in the body, undergoing rapid cellular and vascular changes in order to fulfill its role. B-Mode and Doppler ultrasound has enabled further research into the equine CL and can be utilised as a clinical tool in the assessment of luteal form and function. An understanding of the normal ultrasonographic variations of luteal development and regression is essential to enable clinicians to make an assessment of luteal age as well as make clinical decisions in the gynaecological management of mares under their care. Although advances in the management of brood mares have led to an increase in conception rates, early embryonic death (EED) is still a major economic concern in equine practice. Because EED is in almost all cases recognized retrospectively, it is not surprising that CL resp. progesterone insufficiency is assumed to cause EED. However, there is little scientific evidence to support this hypothesis. Despite preventive managerial measures there are few pharmacological means to improve function of the CL (e.g. Regumate®, GnRH, hCG) in the mare.

Keywords: brood mare, corpus luteum, GnRH, hCG, progesterone.

Resumo

O corpo lúteo (CL) é uma estrutura essencial na regulação do ciclo reprodutor e no estabelecimento de gestação. É um dos órgãos mais vascularizados no corpo, passando por mudanças celulares e vasculares rápidas para realizar sua função. O B-Mode e ultrassom Doppler tem permitido maiores pesquisas no CL equino e pode ser utilizado como ferramenta clínica na avaliação de forma e função lútea. Um entendimento das variações ultrassonográficas normais e desenvolvimento e regressão lúteos são essenciais para permitir que clínicos façam uma avaliação da idade lútea, além de tomar decisões clínicas no gerenciamento ginecológico de éguas sob seus cuidados. Apesar dos avanços no gerenciamento de éguas de reprodução terem levado a um aumento nas taxas de concepção, a morte precoce de embriões (EED) ainda é uma grande preocupação financeira na prática equina. Como o EED em quase todos os casos é reconhecido retrospectivamente, não surpreende que se assume que insuficiência de progesterona de CL responsivo seja a causa de EED. Porém, há pouca evidência científica para suportar esta hipótese. Apesar das medidas preventivas gerenciais, existem poucos meios farmacológicos de melhorar a função do CL (e.g. Regumate®, GnRH, hCG) na égua.

Palavras-chave: égua de reprodução, corpus luteum, GnRH, hCG, progesterona.

Introduction

The luteal gland plays a pivotal role during the estrous cycle and early pregnancy and, therefore, detection and evaluation of the corpus luteum (CL) is critical in providing structural and functional information to assist practitioners on studfarms in comprehending and controlling luteal gland development as well as mitigating potential luteal gland dysfunction (Ginther et al., 2007, Ginther, 2014). Luteal function and fate are controlled by steroid hormones, prostaglandins, nitric oxide, angiogenic and antiangiogenic factors, and numerous cytokines (Niswender et al., 2000, Ferreira-Dias et al., 2006). Early embryonic death (EED) is still a major economic concern in equine practice and is hypothetically assumed to be caused by CL resp. progesterone insufficiency (Allen, 2001). In this review, we discuss imaging of the mare's CL by B-Mode and Doppler ultrasonography, potential factors contributing to EED as well as preventive and pharmacological (e.g. Regumate®, GnRH, hCG) measures to improve function of the CL in the mare.



CL formation in the mare

The CL is a temporary endocrine gland within the ovary that forms by replacing a dominant follicle after ovulation at the end of estrus (primary CL) or during diestrus or early pregnancy (secondary CL) in the mare. Accessory corpora lutea may result from luteinization of anovulatory follicles in estrous mares (hemorrhagic anovulatory follicle, HAF), and especially during early pregnancy from day 35 of gestation onwards.

The primary CL is the principal source of progesterone that regulates estrous behavior and assists in establishing early pregnancy. During the follicular phase progesterone levels are below 1 ng/ml; progesterone begins to rise immediately after ovulation with significant increases occurring within 24 h post ovulation. During the first stage of development (day 0-day 5) of a primary CL progesterone secretion progressively increases accompanied by an increase in CL dimensions and vascularity as well as reorganization of a central blood clot (corpus hemorrhagicum). The equine CL is very sensitive to the luteolytic effects of prostaglandin F_{2α} (PGF_{2α}). The primary CL is responsive - will lyse - to standard-luteolytic doses of prostaglandins from >5 days post ovulation. Progesterone levels peak at ~day 8 followed by a gradual - mare specific - decline until ~day 15. If the mare is not pregnant or spontaneously loses the embryo before approximately day 15 then the CL regresses in response to uterine synthesis and secretion of PGF_{2α} and returns to estrus. Ginther and Santos (2015) described that 40% of the progesterone decrease occurred during a gradual decline between the maximum on day 8 and the beginning of luteolysis on day 15, and 60% occurred during the more abrupt decline following luteolysis. In the absence of luteolysis due to pregnancy, the progesterone concentration at the end of the gradual decline on day 15 is maintained until the beginning of resurgence of the CL of pregnancy at approximately day 35 of gestation. The primary CL is maintained and continues to produce progesterone along with supplemental CL (combined secondary and accessory CL) that forms after day 35 until the placenta becomes the principal source of progesterone starting by day 50 to 70 of gestation. By day 90 to 100 the placenta produces sufficient progesterone and other progestins to maintain pregnancy without any ovarian support. Progesterone is absolutely necessary for the maintenance of pregnancy.

Factors affecting early embryonic death (EED) in the mare

Although advances in the management of brood mares have led to an increase in conception rates, early embryonic death (EED) is still a major economic concern in equine practice (Allen, 2001). To date ~15% of brood mares examined as positive for pregnancy at day 14-16 after ovulation, fail to carry the pregnancy to term. More than 60% of these losses occur before day 42, representing a period when several critical events take place in order to recognize and maintain pregnancy (e.g., maternal recognition of pregnancy, embryogenesis, initial placenta formation, endometrial cup formation). It is mainly the progesterone of the primary CL which supports pregnancy during this critical period. Irvine et al. (1990) measured plasma progesterone concentrations in 179 mares bled on alternate days commencing with a positive pregnancy diagnosis on days 17 to 18 after ovulation and concluding on days 42 to 45. During this period 17 mares (10%) lost their pregnancies, 11 before day 25. In 15 mares the timing of the pregnancy loss could be determined with adequate accuracy; in only one did a decline in progesterone precede the loss. Thus pregnancy loss between days 17 and 42 was rarely caused by a fall in plasma progesterone.

Unfortunately, to date little is known concerning the causes and preventive treatment of EED. Because EED is in almost all cases recognized retrospectively, it is of no wonder that CL resp. progesterone insufficiency is assumed to cause EED. However, there is little scientific evidence to support the theory that administering extra progesterone to a normal mare will either increase the pregnancy rate or decrease the incidence of pregnancy loss.

Factors contributing to EED may be of embryonic, maternal and external "environmental" origin (Allen, 2001). Ultrasonographic characteristic of the embryonic vesicle (e.g. location, size of vesicle and embryo), and chromosomal abnormalities of embryos, gametes of aged mares and stallions, and in stallions and DNA damage of sperm are considered as causative factors with embryonic origin (Rambags et al., 2005; Lear et al., 2008). Maternal factors contributing to EED coincide with insufficient maternal progesterone supply, insufficient endometrial nutrition (e.g. endometrial degeneration, -maldifferentiation), inflammation as a result of persistent post breeding endometritis, and infection, resulting in endometrial prostaglandin release followed by luteolysis and subsequent pregnancy loss. Poor nutrition, systemic disease (e.g. severe colic), stress, pain and transportation are regarded as environmental factors contributing to EED (Van Niekerk et al., 1982; Rambags et al., 2003).

Improving function of the CL in the mare

Progesterone supplementation may be indicated in mares under several clinical conditions: - hastening of the spring transition period, - suppression of estrus behavior, - estrous synchronization, - supplementation



during pregnancy, or - preventative treatment for placentitis and abortion.

Unsurprisingly, mares are often administered exogenous progesterone without any knowledge of their natural blood levels of progesterone following the motto “the more the merrier”. However, mares with a history of repeated pregnancy loss may remain pregnant when supplemented with progesterone.

If the CL does not produce enough progesterone or is destroyed, pregnancy loss may occur. Luteolysis can occur due to local (endometritis) and/or systemic inflammatory processes associated with prostaglandin release.

Controlling progesterone blood levels at critical periods during gestation represents an excellent tool for studfarm practitioners in deciding if progesterone supplementation should start or if continued supplementation is needed. When supplementation during early stages of pregnancy is the task the critical value of the mare’s blood progesterone concentration indicating progesterone supplementation is still a matter of debate. In early pregnancy, progesterone levels above 4.0ng/ml are considered adequate to support pregnancy. Levels below 2.0 ng/ml are considered to initiate supplementation.

Administration is usually initiated within a few days after ovulation and is often continued until day 120 of pregnancy. From day 35 onwards, endometrial cups start to form and secrete equine chorionic gonadotropin (ECG) the latter resulting in formation of secondary CLs. This period of gestation is a good time to measure blood progesterone levels in mares on supplemental progesterone to decide if further supplementation is needed. Ideally progesterone levels in mares on supplemental progesterone should be recontrolled at ~day 60-70. Treatment should be discontinued, if endogenous blood levels of progesterone are determined to be adequate. By day 120-150 of gestation, the placenta takes over the production of various progestagens and progesterone. Ascertaining further need of supplemental progesterone at day 120-150 of gestation is recommended, however good agreement is needed due to varying levels of progestagens in the mare’s plasma showing different cross-reactivities to lab specific progesterone assays. Concentrations of progesterone measured in blood are only 2 to 3 ng/ml during the second half of gestation. This is much lower than levels during the first trimester of pregnancy. Progesterone should not be used for monitoring mid and late trimesters of equine pregnancy, as other progestins maintain the pregnancy.

It goes without saying that general measures under the responsibility of the studfarm veterinarian e.g. good breeding management (e.g. breeding soundness examination, treatment of endometritis, selection of mare with a good fertility prognosis) and reduction of environmental stressors need to be implemented to decrease levels of EED on studfarm.

Progesterone may need to be supplemented generally in early pregnant mares showing estrus signs, with a history of repeated EED, after twin-pitch, in case of endotoxemia and in case of stressful events (e.g. long transportation).

In mares under progesterone supplementation continuation of pregnancy has to be monitored regularly, since many will lose their pregnancy despite supplementation of progesterone and this will prevent those mares returning to estrus.

Several types of progesterone products have been used in an attempt to maintain pregnancies in mares. Regumate[®] is a synthetic progesterone providing the advantage of an oral supplementation to mares. Furthermore, altrenogest (Regumate[®]) will not be measured when determining blood progesterone concentrations, but other sources of progesterone will. The synthetic progestin altrenogest or Regumate[®] and progesterone-in-oil are the only products that will consistently maintain pregnancy in a mare that does not produce sufficient quantities of her own progesterone. Alternative progestagens (injectable hormones) used to prevent pregnancies have been shown in controlled clinical trials to be ineffective in maintaining pregnancy in mares (Daels et al., 1991). Thus, the use of these alternative products cannot be recommended. However, altrenogest will likely suppress endogenous progesterone levels; it has been reported that administration of altrenogest in pregnant mares was associated with lower concentrations of endogenous progesterone from day 14 to day 18 and on day 21 compared with endogenous progesterone levels in pregnant mares not administered altrenogest. This effect was presumed by the authors to be mediated by a reduction in pituitary luteinizing hormone (LH) release and a decrease in luteotropic support (De Luca et al., 2011).

It has been reported, that administration of a single injection of 20-40 µg buserelin (Receptal[®]) between day 9 to day 10 after ovulation produces a significant increase in pregnancy rates, including an increase in embryo numbers in multiple ovulating mares. It was reported that this treatment increased pregnancy rates up to 5-10% (Newcombe et al., 2001; Kanitz et al., 2007). It is evident that buserelin acts independently of the CL (e.g. not by boosting circulating progesterone levels or preventing luteolysis) in the mare (Stout et al., 2002), by limiting the effect of any embryo reduction process operating between day 9 to day 10 and day 13 to day 14 of pregnancy. This may be caused by prevention of EED, which in turn suggests that GnRH could have a direct role at the reproductive tract level that does not involve the ovary. Therefore there could be a direct effect of exogenous GnRH (buserelin) on the relationship between the embryo and the endometrium (Newcombe and Peters, 2014).

In a recent study Köhne et al. (2014) report that hCG injection for induction of ovulation but not on day 5 after ovulation increased progestin concentration in plasma of early pregnant mares. This coincided with an



increased size of the embryo proper respective fetus at the time of the start of placentation. Periovarian treatment of mares with hCG may thus be a valuable tool to enhance conceptus growth during early pregnancy. In this study and in contrast to direct progestin supplementation with altrenogest, hCG treatment stimulated endogenous progesterone secretion. However, Biermann et al. (2014) report that hCG-treatment of mares on day 5 or day 11 post ovulation influenced peripheral progesterone concentrations due to secondary luteal tissue, but did not alter ovarian and uterine blood flow or increase pregnancy rates.

References

- Allen WR.** Luteal deficiency end embryo mortality in the mare. *Reprod Domest Anim*, v.36, p.121-131, 2001.
- Biermann J, Klewitz J, Otzen H, Martinsson G, Burger D, Meinecke-Tillmann S, Sieme H.** The effect of hCG, administered in diestrus, on luteal, ovarian and uterine blood flow, peripheral progesterone levels and pregnancy rates in mares. *J Equine Vet Sci*, v.34, p.166, 2014.
- Daels PF, Stabenfeldt GH, Hughes JP, Odensvik K, Kindahl H.** Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. *Am J Vet Res*, v.52, p.282-228, 1991.
- DeLuca CA, McCue PM, Patten ML, Squires EL.** Effect of a nonsurgical embryo transfer procedure and/or altrenogest therapy on endogenous progesterone concentration in mares. *J Equine Vet Sci*, v.31, p.57-62, 2011.
- Ferreira-Dias G, Costa AS, Mateus L, Korzekwa A, Redmer DA, Skarzynski DJ.** Proliferative processes within the equine corpus luteum may depend on paracrine progesterone actions. *J Physiol Pharmacol*, v.57, suppl.8, p.139-151, 2006.
- Ginther OJ, Gastal EL, Gastal MO, Utt MD, Beg MA.** Luteal blood flow and progesterone production in Mares. *Anim Reprod Sci*, v.99, p.213-220, 2007.
- Ginther OJ, Santos VG.** Natural rescue and resurgence of the equine corpus luteum. *J Equine Vet Sci*, v.35, p.1-6, 2015.
- Ginther OJ.** How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. *Theriogenology*, v.81, p.112-125, 2014.
- Irvine CHG, Sutton P, Turner JE, Mennick PE.** Changes in plasma progesterone concentrations from days 17 to 42 of gestation in mares maintaining or losing pregnancy. *Equine Vet J*, v.22, p.104-106, 1990.
- Kanitz W, Schneider F, Hoppen H-O, Unger C, Nürnberg G, Becker F.** Pregnancy rates, LH and progesterone concentrations in mares treated with a GnRH agonist. *Anim Reprod Sci*, v.97, p.55-62, 2007.
- Köhne M, Ille N, Erber R, Aurich C.** Treatment with human chorionic gonadotrophin before ovulation increases progesterone concentration in early equine pregnancies. *Anim Reprod Sci*, v.149, p.187-193, 2014.
- Lear TL, Lundquist J, Zent WW, Fishback jr WD, Clark A.** Three autosomal chromosome translocations associated with repeated early embryonic loss (REEL) in the domestic horse (*Equus caballus*). *Cytogenet Genome Res*, v.120, p.117-122, 2008.
- Newcombe JR, Martinez TA, Peters AR.** The effect of the gonadotropin-releasing hormone analog, buserelin, on pregnancy rates in horse and pony mares. *Theriogenology*, v.55, p.1619-1631, 2001.
- Newcombe JR, Peters AR.** The buserelin enigma: how does treatment with this GnRH analogue decrease embryo mortality? *J Vet Sci Technol*, v.5, p.151, 2014. doi:10.4172/2157-7579.1000151.
- Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW.** Mechanisms controlling the function and life span of the corpus luteum. *Physiol Rev*, v.80, p.1-29, 2000.
- Rambags BPB, Colenbrander B, Stout TAE.** Early pregnancy loss in aged mares: probable causes and possible cures. *Pferdeheilkunde*, v.19, p.653-656, 2003.
- Rambags BPB, Krijtenburg PJ, Drie HF, Lazzari G, Galli C, Pearson PL, Colenbrander B, Stout TAE.** Numerical chromosomal abnormalities in equine embryos produced in vivo and in vitro. *Mol Reprod Dev*, v.72, p.77-87, 2005.
- Stout TAE, Tremoleda JL, Knaap J, Daels P, Kindahl H, Colenbrander B.** Mid-diestrus GnRH-analogue administration does not suppress the luteolytic mechanism in mares. *Theriogenology*, v.58, p.567-570, 2002.
- Van Niekerk CH, Morgenthal JC.** Fetal loss and the effect of stress on plasma progestagen levels in pregnant Thoroughbred mares. *J Reprod Fertil Suppl*, n.32, p.453-457, 1982.
-