

Use of Serum Progesterone Determination and Synchronization Protocols in Camelid Breeding Programs

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Reproductive management of any domestic species including Camelids requires significant amount of time, effort, and financial input. The cost and efficiency of the reproductive management program (RMP) has a direct influence on the profitability of the operation. Hence, ovarian synchronization protocols were developed in many domestic species in order to improve pregnancy rates, to minimize the handling of large number of animals, to develop fixed time artificial insemination protocols and to manage donors, recipients in embryo transfer programs. There are few reports in the literature using such synchronization protocols in Camelids (Chaves et al., 2002; Ratto et al., 2003; Skidmore et al., 2009). However, these protocols have not been widely applied in practice. In addition, fixed-time, easy to apply and well established breeding/mating protocols with documented fertility data are not available for breeders.

In order to develop and apply such breeding protocols successfully, the thorough understanding of reproductive physiology of these species is indispensable. The aim of this presentation is to summarize the basics of Camelid reproductive physiology, to introduce different synchronization protocols and to demonstrate the use of progesterone determination in Camelid RMPs.

Camelids are induced ovulators (Tibary and Anouassi, 1997). It means that ovulation is not spontaneous; it is induced by a natural stimulus (mating). Ovulation can also be induced artificially by exogenous hormones such as GnRH or hCG. Without such stimuli, ovulation usually does not occur. There are, however, some exemptions from this rule (Nagy et al., 2005).

Follicular development occurs in a waves-like pattern in Camelids. As a result of hormonal stimulus (FSH), a cohort of small follicles emerges on the ovary at regular interval. One of these follicles becomes dominant, continues growing while it suppresses the growth of other follicles in the cohort. Without mating, the so-called dominant follicle undergoes several developmental stages such as growth, mature and regression phases. When this follicle loses its dominance, a next follicular wave starts emerging (Adams 1999; Chaves et al., 2002). The period between emergences of two consecutive follicular waves is called the inter-wave interval. As the dominant follicle reaches 0.7-0.9 cm in diameter, it becomes sensitive to ovulation induction stimuli and remains responsive until reaching the regression phase. If mating or ovulation induction occurs during this time, the dominant follicle ovulates 27-30 hours after the stimulus.

Following ovulation, a corpus luteum (CL) starts developing that has a short lifespan in non-pregnant Camelids. The CL increases in size until Day 8 reaching 1.4-1.9 cm in diameter, then it starts regressing by Day 10 and disappears completely by Days 13-14 after ovulation. The progesterone (P4) production of the CL follows a similar pattern. Serum progesterone concentration becomes detectable by Days 4-5, reaches its peak of 2-5 ng/ml by Day 8, then it returns to baseline level by Days 10-11 (Adams et al., 1991; Nagy et al., 2005). In pregnant Camelids, the CL has a long lifespan: it is morphologically detectable and produces progesterone (serum P4 > 1 ng/ml) throughout the gestation period. In fact, progesterone produced by this CL

is responsible alone for the maintenance of pregnancy in Camelids. For this reason, the determination of serum progesterone concentration has a great diagnostic value in RMPs of Camelids.

Serum progesterone concentration has been determined by various methods in animal reproduction (Nagy et al., 1998). In the Eighties, radio-immunoassays (RIA) were developed that required special laboratory set-up, equipment and laborious sample preparation (extraction). Since then, more practical, easy to use methods were established such as micro-plate enzyme-linked immuno-assays (ELISA), qualitative ELISA (tube test, animal side test) that are available in commercial kits. All these methods are based on the detection of antigen-antibody reaction. Progesterone molecules (antigen) in the serum are bound to specifically raised and labeled antibody molecules in the test system. Labels are radio-isotopes and enzymes in RIA and ELISA systems, respectively. The enzyme in the ELISA converts substrate molecules that results in color reaction. The intensity of this color reaction is proportional to the level of progesterone in serum and can be measured with a photometer (micro-plate reader). In spontaneously ovulating species (cows, horses, dogs etc.), high level ($> 1-2$ ng/ml) of progesterone in serum is an indicator of cyclic ovarian activity (active CL) or pregnancy. In induced ovulators (domestic and wild cats, rabbits, Camelids etc.), naturally serum progesterone is only elevated if the animal is pregnant.

The principal of any ovarian synchronization protocols is the removal of the suppressive effect of the dominant follicle in order to elicit the emergence of a new follicular wave. This can be achieved either by physical or hormonal methods. The physical method, follicular ablation using ultrasound-guided aspiration technique is effective but time-consuming, requires expensive equipment and significant expertise. Hence, it has not been widely used in practice (Ratto et al. 2003; Skidmore et al., 2009). In cattle, several hormone protocols such as Ovsynch, Presynch, Cosynch etc. have been developed and are applied world wide. In Camelids, hormonal treatments for follicular synchronization include the use of GnRH, hCG, LH and steroids (progesterone alone or in combination with 17β -estradiol). The 1st three hormones induce ovulation, while steroids cause regression of the dominant follicle. For synchronization of follicular wave emergence, ovulation induction by hormone seems to be the most efficient as well as practical method. In general, a random GnRH injection induces ovulation in 50 to 60 % of the animals. Repeated GnRH treatments with or without $PGF_{2\alpha}$ could result in 75 to 90 % ovulation rate, but fertility after such synchronization protocol has not been documented yet (Skidmore et al., 2009). In our studies with lactating dromedaries, per-cycle pregnancy rate is 45 to 55 % after ovarian synchronization and fixed-time natural mating.

We can conclude that an ovarian synchronization protocol - followed by fixed-time natural mating and combined with serum progesterone determination to monitor ovulation and diagnose pregnancy - provides an excellent and practical tool in reproductive management programs of Camelids in order to minimize efforts and maximize breeding efficiency.

Reference

Adams, G.P.: Comparative patterns of follicle development and selection in ruminants. J. Reprod. Fertil. Suppl. 1999;54.17-32.

Adams, G.P., Sumar, J., Ginther, O.J.: Form and function of the corpus luteum in llamas. *Anim.Reprod.Sci.*, 1991.24.127-138.

Chaves, M.G., Aba, M., Agüero, A., Egey, J., Berestin, V., Rutter, B.: Ovarian follicular wave pattern and the effect of exogenous progesterone on follicular activity in non-mated llamas. *Anim. Reprod.Sci.*, 2002.69.37-46.

Nagy, P., Juhasz, J., Wernery, U.: Incidence of spontaneous ovulation and development of the corpus luteum in non-mated dromedary camels (*Camelus dromedarius*). *Theriogenology*, 2005.64.292-304.

Nagy, P., Solti, L., Kulcsár, M., Reiczigel, J., Huszenicza, GY., Abaváry, K.M., Wölfling, A.: Progesterone determination in equine plasma using different immunoassays. *Acta Veterinaria Hungarica*, 1998.46(4).501-513.

Ratto, M.H., Singh, J., Huance, W., Adams, G.P.: Ovarian follicular wave synchronization and pregnancy rate after fixed-time natural mating in llamas. *Theriogenology*, 2003.60.1645-1656.

Skidmore, J.A., Adams, G.P., Billah, M.: Synchronisation of ovarian follicular waves in the dromedary camel (*Camelus dromedarius*). *Anim. Reprod.Sci.*, 2009.114 (1-3).249-255.

Tibary, A., Anouassi, A.: Reproductive physiology in the female camelidae. In: *Theriogenology in Camelidae*. Institute Agronomique et Veterinaire Hassan II, Rabar, Maroc, 1997.169-241.

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