

## **EARLY EMBRYONIC DEVELOPMENT AND MATERNAL RECOGNITION OF PREGNANCY IN CAMELIDS: A REVIEW**

Desarrollo embrionario temprano y el reconocimiento materno de la preñez en los camélidos:  
Una revisión

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### **ABSTRACT**

Maternal recognition of pregnancy (MRP) is a biological process that allows a female to recognize the presence of a conceptus and sustain the lifespan of the corpus luteum (CL). In-depth studies on MRP have been sparked by the impact of early pregnancy loss on productivity in various large animal domestic species. Reproductive studies in camelids are limited and our knowledge of MRP in this species has lagged behind compared to other domestic species. MRP in camelids seems to be driven primarily by estrogen secretion from the hatched blastocyst and possibly other mechanisms related to elongation. The presence of the conceptus blocks the induction of endometrial COX-2 expression resulting in attenuation of PGF2 $\alpha$  and rescue of the CL. A substance such as IFNT has not been identified in camelids. Oxytocin does not appear to play a major contributing role in this process. Embryos migrate to the left uterine horn in order for luteal function to be maintained. Studies thus far have not shown a difference between the left and right uterine horn related to histological changes and estrogen receptor expression. More recently investigation of expression levels of genes previously identified as conceptus regulated genes in the endometrium of other domestic animals has been evaluated in camels. The objectives of the current review are to present the current state of our knowledge on MRP in camelids and discuss early embryonic development in this species and how it may relate to MRP.

**Keywords:** camelid, pregnancy, embryo, corpus luteum, MRP

### **RESUMEN**

El reconocimiento materno de la preñez (MRP) es un proceso biológico que permite a hembra reconocer la presencia de un embrión y sostener la vida del cuerpo lúteo (CL). Los estudios en profundidad sobre MRP han sido ocasionados por el impacto de la pérdida temprana de la preñez en la productividad de varias especies domésticas de animales granja. Los estudios reproductivos en camélidos son limitados y nuestro conocimiento de MRP en esta especie se ha retrasado en comparación con otras especies domésticas. El MRP en los camélidos parece estar causado principalmente por la secreción de estrógenos del blastocisto y posiblemente otros mecanismos relacionados con su elongación. La presencia del embrión bloquea la inducción de la expresión de COX-2 endometrial que resulta en la atenuación de PGF2 $\alpha$  y el rescate del CL. Una sustancia tal como IFNT no ha sido identificada en los camélidos. La oxitocina no parece jugar un papel importante en este proceso. Los embriones migran hacia el cuerno uterino izquierdo para mantener la función lútea. Hasta el momento, los estudios no han mostrado una diferencia entre el cuerno uterino izquierdo y el derecho en relación con los cambios histológicos y la expresión del receptor de estrógenos. Más recientemente, se ha evaluado en camellos la investigación de los niveles de expresión de los genes identificados previamente como genes regulados por el embrión en el endometrio de otros animales domésticos. Los objetivos de esta revisión son presentar el estado actual de nuestro conocimiento sobre MRP en camélidos y analizar el desarrollo embrionario temprano en esta especie y cómo se puede relacionar con MRP.

**Palabras clave:** Camélidos, preñez, embrión, cuerpo lúteo, MRP

## INTRODUCTION

Maternal recognition of pregnancy (MRP) is the biological process that allows a female to recognize the presence of a conceptus and remodel the endocrine, histological, and secretory function within the reproductive tract to allow attachment/implantation and further embryonic and fetal development. This process, which can be summarized as a mechanism of prevention of luteolysis, involves signaling between the embryo and the uterus leading to successful attachment/implantation. In-depth studies on MRP have been sparked by the impact of early pregnancy loss on productivity in various species such as cattle (Diskin and Morris, 2008), camelids (Fernandez-Baca et al., 1970; Tibary et al., 2007; Tibary and Pearson, 2015), and horses (Allen et al., 2007; Ball and Woods, 1987; Canisso et al., 2013; Vanderwall, 2008). Additionally, early embryonic loss (during the pre-implantation period) is a common problem with many advanced reproductive biotechnologies involving embryo/gamete manipulations. This increased early embryonic loss is generally attributed to impaired communication between the developing blastocyst and the maternal endometrium (Farin et al., 2010). In the past three decades, tremendous advances have been made in deciphering some of the MRP signaling particularly in ruminants (Sandra et al., 2015; Spencer et al., 2007; Spencer et al., 2008), swine (Bazer and Johnson, 2014; Franczak and Bogacki, 2009; Jalali et al., 2015) and to a lesser extent in horses (Katila, 2011; Klein and Troedsson, 2011a, b).

Camelidae (or camelids) are economically important in South America, Africa, Asia and the Middle East. However, they have also become increasingly popular in other parts of the world including North America. They are one of the only production animal species that can survive under some of the harshest climatic conditions on the planet. Reproductive capacity is an important component of production and profitability within the camelid industry and breeding management strategies can be challenging.

Pregnancy loss is the second most common complaint in camelid reproduction (Pearson et al., 2014; Tibary et al., 2007; Tibary and Pearson, 2015; Verdier and Bornstein, 2010). Epidemiological data on the incidence of pregnancy loss in camelids is scarce (Knight et al., 1995; Tibary, 2010; Vaughan and Tibary, 2006). Early embryonic death can reach up to 50% in naturally mated females and between 20 and 60% in embryo transfer recipients (Anouassi and Tibary, 2010). There are several contributing factors to early embryonic loss. Season of mating was shown to be an important factor in alpacas. Pregnancy loss prior to 81 days of gestation is higher in autumn-bred females (17.3%) than in spring-bred females (2.8%) (Knight et al., 1995). In camels, pregnancy loss can reach alarming levels particularly when nutrition is not adequate or during outbreaks of diseases such as trypanosomiasis (Tibary et al., 2005; Tibary and Anouassi, 1996).

Pregnancy loss may also be due to age-related or pathologic changes to the endometrium. Histological evaluation of endometrial biopsies has long been recognized as an important method for evaluation of infertility and breeding soundness in mares. Although a grading system for histological evaluation has been proposed in camelids (Powers et al., 1990), it is not widely used in a clinical setting. A better understanding of normal histological changes associated with the development of early pregnancy would improve our understanding of pathologic conditions of the reproductive tract that may lead to infertility or early embryonic loss. As in other species, oocyte

quality, male factors, and environmental factors may be involved in the occurrence of pregnancy loss.

Studies on early embryonic development and MRP in camelids are scarce compared to other large animal domestic species. The objectives of this paper are to present the current state of our knowledge on MRP in camelids and discuss early embryonic development in camelids and how it may relate to MRP. Throughout the paper unless a specific species is mentioned, the term South American Camelids (SAC) will be used to refer to alpacas (*Vicugna pacos*), llamas (*Lama glama*), Guanacos (*Lama guanacoe*) and vicuñas (*Vicugna vicugna*). The term camels will refer to all old-world camelids (*Camelus dromedarius* and *Camelus bactrianus*). The term camelids will be used to include all of these species.

## 1. Early embryonic development in camelids

The physiology of pregnancy in camelids presents several peculiarities compared to that of other domestic species. Knowledge of embryonic development, fetal development and characteristics of placentation are fundamental not only for diagnosis of pregnancy and fetal evaluation but also for clinical and pathological evaluation in cases of pregnancy loss or abortion. In addition, the understanding of early embryo development and mechanisms of MRP are important for developing therapeutic strategies to prevent early embryonic death, as well as progressing advanced reproductive techniques such as embryo transfer technologies (Tibary et al., 2007).

### 1.1 Ovulation and fertilization

Camelids are induced ovulators. Follicular waves overlap continuously in the absence of mating (Skidmore, 2011; Tibary et al., 2007; Vaughan, 2011). The preovulatory luteinizing hormone (LH) surge occurs after mating in response to an ovulation inducing factor (OIF) in seminal plasma. Recently, this OIF has been identified as  $\beta$  nerve growth factor ( $\beta$ NGF) (Adams and Ratto, 2013; Kershaw-Young et al., 2012; Kumar et al., 2013). It is important to note that  $\beta$ NGF may have a luteotropic effect on the corpus luteum (CL) (Silva et al., 2014). Oocyte maturation is completed immediately following copulation and LH release and shortly after ovulation, which occurs around 28 to 32 hours after copulation. The LH surge triggers resumption of meiosis in the oocyte, disruption of cumulus cell cohesiveness and rupture of the follicular wall (Vaughan, 2011). Cumulus dispersion is observed in cumulus-oocyte complexes (COC) recovered by aspiration from follicles 18 to 24 hours after human chorionic gonadotropin (hCG) injection (Del Campo et al., 1995; Tibary et al., 2007).

Both ovaries are equally active and alternance of ovulation between ovaries occurs occasionally (Campbell et al., 2015; Tibary et al., 2007). Double ovulations are not uncommon in most domestic camelids in good health and nutritional status (Campbell et al., 2015). Triple and quadruple ovulations have also been documented in the dromedary camel (Tibary and Anouassi, 1996).

Semen is deposited deep into the uterine horns throughout the mating period and slowly forms a spermatozoa reservoir in the uterine tube as it liquefies (Bravo et al., 1996; Tibary et al., 2007; Vaughan and Tibary, 2006). The molecular and ultrastructural aspects of spermatozoa-oviductal attachment and reservoir formation have been investigated in llamas. These studies demonstrated the role played by the bulbourethral gland secretions in this phenomenon (Apichela et al., 2009; Apichela, 2011; Apichela et al., 2014; Apichela et

al., 2010). Spermatozoa may be stored in the isthmus region for up to 5 days (Stekleniov, 1968; Thibault, 1973; Tibary et al., 2007).

Fertilization rates are very high (>80%) in alpacas (Campbell et al., 2015). Some authors have observed higher fertilization rates in alpacas following ovulation from the left ovary than from the right ovary (Fernandez-Baca et al., 1970). However, this difference has not been observed in our laboratory in camels and alpacas (Campbell et al., 2015; Picha et al., 2013; Tibary and Anouassi, 1996). A similar observation was reported by Ratto et al., 2011 in alpacas. Conception rates per mating are generally lower when females are mated based on receptivity rather than when mating is based on presence of a mature follicle (Campbell et al., 2015; Gupta et al., 1978; Tibary et al., 2007). However, in well-managed herds conception rates are generally high (>90%) (Campbell et al., 2015). There is a tendency for fertilization rate and embryo survival to be lower when females are mated during the regression phase of the follicular wave (Ratto et al., 2011). However, the difference is not statistically significant. One of the main challenges to reproductive efficiency in camelids is the high incidence of early pregnancy loss. Embryo mortality rates of up to 35% has been documented in alpacas in the first 35 days after mating (Bravo et al., 2010).

### 1.2 Early embryonic development within the uterine tube

Early stages of embryo segmentation take place in the uterine tube (Picha et al., 2013; Tibary et al., 2007). Studies conducted in our laboratory on camels and alpacas show that the development of the embryo from fertilization to the hatched blastocyst stage takes about 6 to 6.5 days following ovulation and fertilization (day 7.5 after mating). The embryo reaches the uterus around this time, at the hatched expanding blastocyst stage (Adam et al., 1992; Bourke et al., 1992; McKinnon et al., 1992; Picha et al., 2013; Tibary, 2010; Tibary et al., 2007). There does not seem to be a selective transport through the uterotubal junction (UTJ) as seen in the mare. Unfertilized ova degenerate or are eliminated in the uterine cavity soon after ovulation (Picha et al., 2013). The significance of this early hatching of the blastocyst as it relates to MRP has not been investigated.

### 1.3 Embryo hatching, elongation, and migration

The hatched blastocyst expands promptly and starts to elongate on day 9 growing rapidly to occupy the entire uterine cavity by day 12. By day 15 post-ovulation, the trophoblast is in contact with the entire endometrial surface although not yet attached (Picha et al., 2013; Tibary et al., 2007). During the process of elongation, the embryo migrates from the right uterine horn to the left uterine horn for females that had a right-sided ovulation. In alpacas, 83.3% of the embryos resulting from right ovarian ovulations were found in the left uterine horn on day 9 (Picha et al., 2013). Camelids are unique in that greater than 98% of pregnancies are carried in the left uterine horn (Tibary et al., 2007). This suggests that embryo migration to the left horn is an important mechanism in prevention of luteolysis in these species.

These observations are supported by embryo transfer experiments. In llamas, transfer of embryos to the uterine horn ipsilateral to the ovulation side resulted in higher pregnancy rates for left ovulation (50%) than right ovulation (30%) recipients, while transfer of the embryo to the left uterine horn when the ovulation (mature CL) was on the right ovary resulted in only 20% pregnancy rate (Trasorras et al., 2010). In alpacas, transfer of embryos collected at 7 days and 4 hours

into the left horn, regardless of the side of ovulation in the recipient, resulted in higher pregnancy rate (20.3%) when the CL was on the left ovary than when the CL was on the right ovary (12.4%) (Picha et al., 2010). In the dromedary, deposition of the embryo in the right uterine horn when the CL was on the right ovary resulted in higher pregnancy rates compared to left horn transfer when the CL was on the right ovary (Anouassi and Tibary, 2010).

## 2. Maternal recognition of pregnancy in camelids

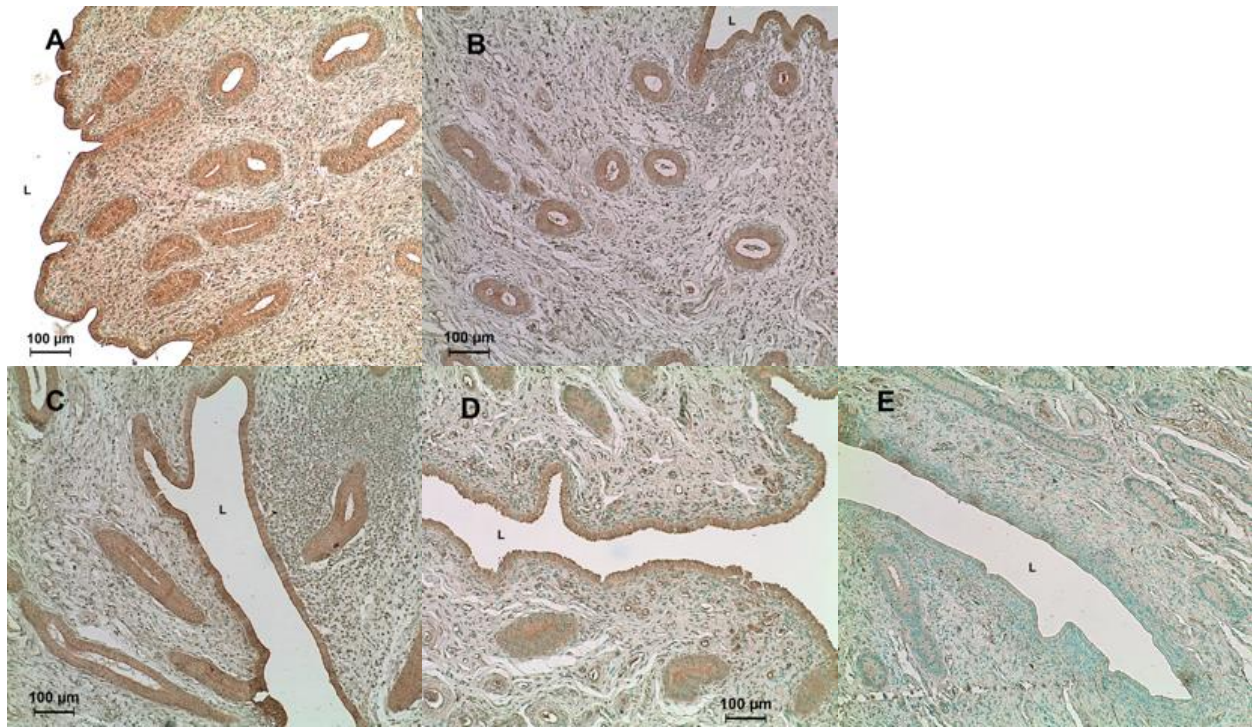
In the absence of pregnancy, the luteal phase in camelids is very short. The CL develops after ovulation and reaches its maximal size and function (progesterone production) by day 8 to 9 post-mating. The CL is necessary for maintenance of pregnancy throughout gestation. Luteolysis occurs in response to increased pulsatile prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\alpha$ ) secretion from the endometrium and is complete by day 10 or 11 post-ovulation. In the presence of pregnancy, luteolysis is prevented and the CL is maintained as the sole source of progesterone throughout gestation (Adams et al., 1991; Smith et al., 2000; Tibary et al., 2007). The size of the CL varies between 11 and 20 mm in SAC and 15 to 25 mm in camels. A cavitory CL is normal (Tibary et al., 2007). Recent studies have shown that a CL resulting from an ovulation induced by seminal plasma (i.e. mating to a vasectomized male) is larger and produces more progesterone than those emanating from induction of ovulation with GnRH (Silva et al., 2014).

Unlike ruminants, an increase (or a cyclical regulation) in the endometrial oxytocin receptor (OTR) population before and during luteolysis could not be observed in llamas (Bianchi et al., 2010). Therefore, changes in the population of oxytocin receptors does not seem to be involved in the initiation of luteolysis in llamas. A previous study on dromedaries showed that a single dose of oxytocin administered 10 days post-ovulation did not result in a release of PGF<sub>2</sub> $\alpha$  (Skidmore et al., 1998). Recently, we demonstrated that exogenous administration of oxytocin (20 or 60 IU, IM daily) from day 3 to day 10 post-induction of ovulation did not influence the duration of CL activity or progesterone concentrations. All alpacas (experimental and control) underwent luteolysis at the expected time (by day 10 post-ovulation) indicating that oxytocin was not involved in the initiation of early luteolysis in alpacas (Ciccarelli et al., 2017). Further, expression of OTR between pregnant and non-pregnant alpaca uteri around the time of MRP or completion of embryo elongation did not reveal any significant differences (Figure 1). These results suggest that oxytocin likely does not play a major role in luteolysis or MRP, and that other mechanisms are most likely involved in inhibition of PGF<sub>2</sub> $\alpha$  release and prevention of luteolysis in pregnant alpacas (Ciccarelli et al., 2017).

Clinical and endocrine studies suggest that MRP in camelids has to take place relatively early after mating (between day 7 to day 10) in order to prevent luteolysis. Despite the presence of a conceptus, a pulsatile release of PGF<sub>2</sub> $\alpha$  is observed between day 7 and day 15 post-mating. This release of PGF<sub>2</sub> $\alpha$  results in a transient decrease in progesterone concentrations by day 9 post-mating, with a subsequent recovery in progesterone concentrations (Aba et al., 1997; Aba et al., 2000). During the same period, endometrial expression of cyclooxygenase-2 (COX-2) in the luminal epithelium decreases, reaching one-third the level observed during luteolysis in non-pregnant animals by day 12 (Bianchi, 2011). Bianchi et al. showed a reduction in COX-2 immunostaining (both percentage of positive cells and the intensity of staining) in the luminal epithelium between pregnant llamas 12 days-post mating and non-pregnant llamas

12 days post-induction of ovulation (Bianchi et al., 2015). Furthermore, there was a reduction in COX-2 immunostaining from day 8 to day 12 post-mating in pregnant llamas. This low expression of COX-2 in the endometrium of pregnant llamas by day 12 post-mating could explain the attenuation of PGF2 $\alpha$  previously reported in pregnant llamas (Powell et al., 2007b). These findings are similar to those reported in horses, where the presence of the conceptus blocks the induction of endometrial COX-2 expression, suggesting that this is a mechanism by which

luteolysis is prevented during early pregnancy (Bianchi et al., 2015). It is important to note that MRP occurs at a time when the embryo undergoes a rapid elongation as observed in ruminants. However, attempts to identify in camelids a substance such as interferon tau (IFN $\tau$ ) in ruminants were not successful (Abdoon et al., 2017; Skidmore et al., 1994).



**Figure 1.** Immunohistochemical localization of OTR in the endometrium in open (A and B) and pregnant (C and D) alpacas. A) Day 9 post-induction of ovulation, B) Day 14 post-induction of ovulation, C) Day 9 post-mating, D) Day 14 post-mating, E) negative control. L = lumen (100x)

Similarly, to the porcine and equine embryo, camelid embryos have a high aromatizing ability producing estradiol-17 $\beta$  between days 10 and day 15 of pregnancy (Powell et al., 2007b; Skidmore et al., 1994). There is a temporal relationship between an increase in estradiol synthesis by the embryo and MRP. The involvement of estrogens in MRP in camelids is further supported by the increase in estrogen receptor  $\alpha$  (ER $\alpha$ ) between days 8 to 12 post-mating in pregnant animals and a reduction in the expression of progesterone receptor (PGR) by day 12 post-mating (Bianchi, 2011; Bianchi et al., 2015). Recently increased expression of ER $\alpha$  in the stromal cells on day 8 post-mating in pregnant llamas and in the luminal epithelium by day 12-post mating is suggestive that estrogen release from the blastocyst during early development could be stimulating changes in the uterine function necessary for maintenance of pregnancy (Bianchi et al., 2015). Additionally, administration of estradiol benzoate (10 mg, IM) to llamas from day 7 to day 15 after induction of ovulation with hCG, resulted in an extension of the CL lifespan and progesterone production (Powell et al., 2007b). In alpacas, administration of estradiol on days 8 and 9 post-ovulation improved embryo survival by 30 to 50% (Chipayo, 2003; Palomino et al., 2006). However, a similar protocol in

llamas resulted in a decrease in pregnancy rate (Trasorras et al., 2011).

The decrease in PGR in the luminal and glandular epithelium by day 12 post-mating in pregnant llamas agrees with similar findings reported in most domestic animals prior to attachment/implantation, and could be necessary to allow expression of proteins involved in attachment/implantation of the embryo (Bianchi et al., 2015). Abdoon et al. 2017 demonstrated a significant decrease in PGR gene expression in the left and right uterine horn of pregnant compared to non-pregnant dromedary camels at day 10 post-mating or post-induction of ovulation respectively.

The time of embryo attachment is not well defined in camelids. Although early studies have suggested that the embryo remains free within the uterus for the first 30 days (Bravo and Sumar, 1981), recent studies suggest that attachment may start as early as 14 days of pregnancy. One of the major peculiarities of embryo fixation and pregnancy in camelids is that nearly all fetuses are located in the left uterine horn (Bravo and Varela, 1993; El Wishy et al., 1981; Fernandez-Baca et al., 1973; Fernandez-Baca et al., 1974). This situation predominates (98 to 100%) in all camelids although the location of the CL is equally distributed between the left and right ovary (Table 1).



A higher incidence of right horn pregnancies (40%) was reported in one study in the dromedary (Hussein et al., 1991). These authors reported an incidence of right horn pregnancies of 30 to 60% even for stages of pregnancy beyond 95 days. The discrepancy between the results of this particular study and those of all other published studies on this topic raises some doubt as to their validity. In one study on alpacas, only one fetus out of 48 (2%) was located in the right uterine horn at

120 days of pregnancy (Bravo and Varela, 1993). In our clinic, only 4 out of 640 pregnancies were diagnosed in the right uterine horn between 25 and 40 days of pregnancy and all resulted in an early loss of pregnancy before 60 days. The side of ovulation does not influence pregnancy rate (Ratto et al., 2011; Tibary and Anouassi, 1996).

**Table 1.** Distribution of ovulation between left and right ovary and incidence of left horn pregnancies in camelids

Species	Left ovary (%)	Right ovary (%)	Pregnancies in the left horn (%)	Reference
<i>C. dromedarius</i>	52.7	47.3	100.0	(El Wishy et al., 1992)
	53.1	46.9	100.0	(El Wishy and Elsawaf, 1971)
	50.2	49.8	99.0	(Musa and Abu Sineina, 1976a, b)
	56.5	43.5	99.24	(Shalash, 1965)
	47.3	52.7	100.0	(Shawki et al., 1992)
	54.7	45.3	98.2	(Ghoneim, 1985)
<i>V. pacos</i>	50.0	50.0	98.0	(Bravo and Varela, 1993)
<i>L. glama</i>	50.0	50.0	98.4	(Sumar, 1984)

The preponderance of left-horn pregnancies in camelids has been attributed to a difference in PGF2 $\alpha$  release between the two uterine horns. PGF2 $\alpha$  release from the right uterine horn is local, only affecting the right ovary, whereas its release from the left horn is systemic, affecting both ovaries (Tibary et al., 2007). Removal of the left horn prolongs luteal activity (CL lifespan) on either ovary. Conversely, removal of the right uterine horn in the presence of a right ovarian CL causes only a slight delay in luteal regression demonstrating that the right uterine horn has only local luteolytic effect. The migration of the embryo originating from an ovulation in the right ovary to the left horn is therefore required to prevent PGF2 $\alpha$  release into the general circulation and may even exert a luteotrophic effect making possible the survival of the embryo (Fernandez-Baca et al., 1973; Picha et al., 2013; Tibary et al., 2007).



**Figure 2.** The gross anatomy of the uterus from an adult alpaca: right horn (RH), left horn (LH), body (B), cervix (C), uterine tube (UT), O (ovary), and broad ligament (BL).

The exact mechanism of embryo migration is not known. It has been suggested that embryo migration is facilitated by the shortness of the uterine body, the smallness of the right horn, and the increased number of mucosal folds in the endometrium

of the right horn (Musa and Abu Sineina, 1976b). Gross examination of the uterus from fetal, pre-pubertal, and non-pregnant female camelids has demonstrated that the left uterine horn is consistently larger when compared to the right uterine horn (Figure 2) (Porjoosh et al., 2010; Vaughan and Tibary, 2006). Mobility of the embryo as observed in the horse is less likely to happen in camelids, because of the elongation of the blastocyst and the decrease in tone and contraction of the uterus during pregnancy.

**Table 2.** Mean (Mean $\pm$ SE) of the endometrial and myometrial thickness of the left (LH) and right (RH) uterine horn in 10 pregnant and 10 non-pregnant alpacas (Campbell, 2015)

Thickness (mm)	Pregnant Day 9	Open Day 9	Pregnant Day 14	Open Day 14
RH Endometrium	2.91 $\pm$ 0.20 <sup>a</sup>	2.91 $\pm$ 0.25 <sup>a</sup>	3.06 $\pm$ 0.17 <sup>a</sup>	2.17 $\pm$ 0.19 <sup>b</sup>
LH Endometrium	3.46 $\pm$ 0.38 <sup>a</sup>	3.27 $\pm$ 0.37 <sup>a</sup>	3.13 $\pm$ 0.44 <sup>a</sup>	2.53 $\pm$ 0.13 <sup>b</sup>
RH Myometrium	3.36 $\pm$ 0.41 <sup>a</sup>	2.58 $\pm$ 0.37 <sup>a</sup>	3.76 $\pm$ 0.44 <sup>a</sup>	2.75 $\pm$ 0.19 <sup>b</sup>
LH Myometrium	3.44 $\pm$ 0.34 <sup>a</sup>	2.78 $\pm$ 0.17 <sup>a</sup>	4.11 $\pm$ 0.46 <sup>a</sup>	2.58 $\pm$ 0.40 <sup>b</sup>

<sup>a,b</sup> Significance was set at P < 0.05

**Table 3.** Mean (Mean $\pm$ SE) of the endometrial to myometrial (E:M) ratio of the left and right uterine horn in 10 pregnant and 10 non-pregnant alpacas (Campbell, 2015)

E:M Ratio	Pregnant Day 9	Open Day 9	Pregnant Day 14	Open Day 14
Left Uterine Horn	0.50 $\pm$ 0.10	0.54 $\pm$ 0.44	0.43 $\pm$ 0.03	0.51 $\pm$ 0.04
Right Uterine Horn	0.47 $\pm$ 0.10	0.52 $\pm$ 0.05	0.45 $\pm$ 0.04	0.44 $\pm$ 0.13

Our laboratory performed histological evaluation of the left versus right uterine horn in non-pregnant and early pregnant alpacas. Although the vast majority of camelid pregnancies attach within the left uterine horn, histologic structure of the uterus in bilateral horns was similar. Endometrial and myometrial thickness is reported in Tables 2 and 3. Histological evaluation did not show any difference in glandular density and tortuosity, vascularity, or infiltration of inflammatory cells between the left and right uterine horn for females in each experimental group (Campbell, 2015). Additionally, histologic structure of the uterus was similar in the two uterine locations that were sampled (near the tip and at the base of the uterine horn). This is suggestive that there is uniformity in endometrial and myometrial structure throughout the uterus in alpacas. These results agree with previously reported observations in camels (Chen et al., 2003). Glandular tortuosity and increased luminal diameter in both uterine horns were observed occurring between day 9 and day 14 of pregnancy compared to day 14 post-induction of ovulation (Figure 3) and is attributed to the continuing influence of luteal progesterone and increased histotroph production. These changes are consistent with the development of an agreeable environment for embryo survival during early pregnancy (Campbell, 2015).

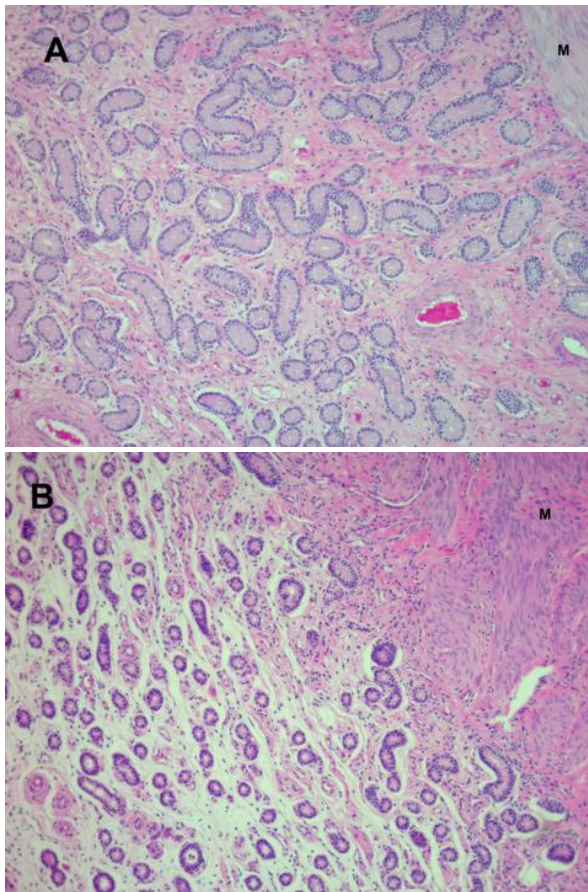


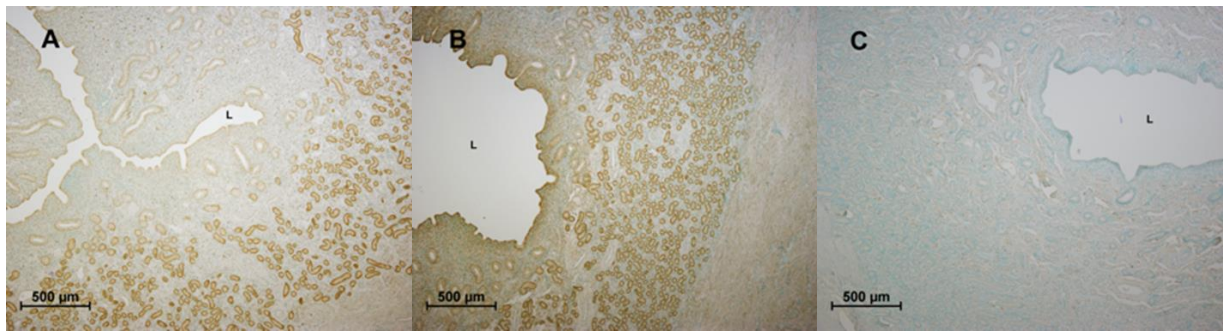
Figure 3. Differences in endometrial glandular tortuosity and luminal diameter A) Day 14 pregnant alpaca, B) Day 14 open alpaca. M=myometrium (H&E; 400x)

Evaluation of uterine fluid from non-pregnant alpacas, pregnant alpacas between 34 to 37 days of gestation, and pregnant alpacas between 60 to 64 days of gestation demonstrated differences between the left and right uterine horn (Argañaraz et al., 2015). The study observed specific variations in protein patterns between the left and right uterine horn associated with signal translation, immunology, glycolysis, angiogenesis, and protection against oxidative stress (Argañaraz et al., 2015). These results strongly suggest that additional studies to evaluate protein patterns of uterine fluid surrounding the timing of MRP are warranted for further understanding of embryo-maternal crosstalk during early pregnancy.

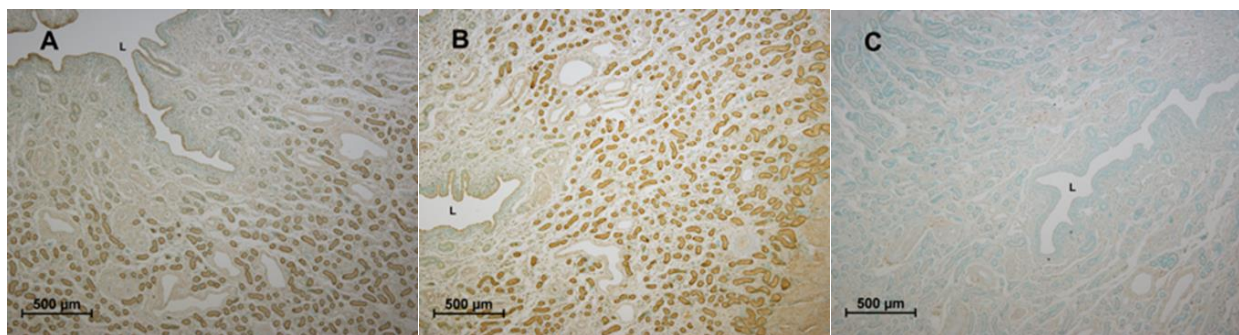
More recently a study in the dromedary camel showed differences in expression of the transcription factor gene STAT1 and interferon stimulated gene OAS1 between the left and right uterine horn during preimplantation and early placentation (Abdoon et al., 2017). The STAT1 mRNA level was higher in the right uterine horn compared to the left uterine horn at day 8 post-mating. By 4 to 6 weeks of gestation the STAT1 mRNA level was significantly higher in the left uterine horn compared to the right uterine horn. OAS1 mRNA levels were lower in the endometrium of the left uterine horn compared to the right uterine horn at day 10 post-mating (Abdoon et al., 2017), showing that conceptus attachment/implantation is not associated with an interferon response in the pregnant uterine horn. These findings demonstrate that endometrial genes are differentially regulated between the left and right uterine horn relative to the presence of a conceptus, however additional research is needed to further establish the molecular mechanisms that drive the establishment and progression of pregnancy in the left uterine horn of camelids (Abdoon et al., 2017).

Estrogen receptor  $\beta$  (ER $\beta$ ) expression was found to be greater in pregnant than in sterile-mated llamas particularly on day 13. The authors suggested that the presence of an embryo may be influencing this expression (Powell et al., 2007a). Endometrial expression of ER $\alpha$  and ER $\beta$  was not affected by uterine side or days post-mating. However, the presence of a CL seems to up-regulate ER $\beta$  expression in the uterus mostly in non-endometrial tissue. While endometrial ER $\beta$  are not affected by pregnancy status, the uterine ER $\beta$  is significantly increased. This led the authors to conclude that the embryo exerts a direct effect on non-endometrial tissue (myometrium and perimetrium). These effects may operate through estradiol secretion. Because all embryos migrate to the left horn, a greater expression of ER in the right horn to promote its contraction was expected but could not be demonstrated. This suggests that a more complex mechanism with differential expression of ER subtypes may be involved in embryo migration from the right to the left horn (Powell et al., 2007a). These findings were supported by recent work in our laboratory, which did not demonstrate a greater expression of ER $\alpha$  in the right uterine horn of pregnant alpacas (Figure 4 and 5) to promote its contraction (Campbell, 2015), as well as recent research performed in camels (Abdoon et al., 2017).





**Figure 4.** Immunohistochemical localization of ER $\alpha$  in the endometrium of Day 9 pregnant alpacas: A) left horn, B) right horn, C) negative control. L = lumen (40x)



**Figure 5.** Immunohistochemical localization of ER $\alpha$  in the endometrium of Day 14 pregnant alpacas: A) left horn, B) right horn, C) negative control. L = lumen (40x)

## CONCLUSION

Maternal recognition of pregnancy and attachment/implantation require very complex interactions between the maternal endometrium and the conceptus. Our knowledge of MRP in camelids lags behind compared to other domestic species, particularly ruminants. MRP in camelids seems to be driven primarily by estrogen secretion from the hatched blastocyst and possibly other mechanisms related to elongation and intimate contact of the trophoblast with the endometrium. A substance such as IFN $\tau$  has not been identified in camelids. The presence of the conceptus blocks the induction of endometrial COX-2 expression resulting in attenuation of PGF2 $\alpha$  and rescue of the CL. However, oxytocin does not appear to be a major contributing factor to luteolysis or MRP as demonstrated by recent research.

The requirement for pregnancies to be maintained in the left uterine horn is unique to camelids and the exact mechanism of embryo migration is still unknown. Studies thus far have shown that there is no difference between the left and right uterine horn related to histological changes and estrogen receptor expression. More recently investigation of expression levels of genes previously identified as conceptus regulated genes in the endometrium of other domestic animals has demonstrated differences between the left and right uterine horn on a molecular level.

Deciphering the mechanism of prevention of luteolysis in camelids is fundamental for furthering our knowledge on causes of early embryonic loss and its prevention. There is a lack of knowledge of molecular mechanisms associated with the crosstalk occurring between the maternal endometrium and the embryo necessary for MRP and early pregnancy development.

Further genomic and proteomic studies evaluating changes in gene expression associated with conceptus regulation and endometrial changes surrounding early embryonic development and attachment/implantation are necessary for further understanding the mechanisms of MRP in this species.

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