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Review paper

Endocrine control of pregnancy and parturition in South American camelids and Old World camels and the resulting possibilities for hormonal pregnancy diagnostics

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Abstract

For South American camelids (SAC) and, to a lesser extent, Old World camels (OWC), an increasing demand for veterinary services has developed in Central Europe in recent years, with specific knowledge of the reproductive endocrinology of this species being particularly in demand for the management of breeding farms. Compared to many other domestic animal species, relatively little reliable information is available on the endocrine control of pregnancy and parturition in camelids. However, some significant differences to other domestic ungulate species are evident. Knowledge of pregnancy-associated endocrine changes forms the basis for hormonal pregnancy diagnostics. Even though clinical pregnancy diagnostics using sonography is also of primary importance in camelids, hormonal methods, especially non-invasive methods, are potentially of considerable interest as they represent a less stressful or stress-free alternative. Non-invasive methods of pregnancy diagnostics are of particular interest in untrained OWC, where clinical diagnostics or blood sampling without sedation can be associated with unacceptable risks for the personnel involved. Experience with hormonal pregnancy diagnostics in camelids has so far only been published sporadically, with mostly progesterone, pregnancy-associated estrogens or relaxin being measured in the blood. Non-invasive measurement of progesterone or estrogen metabolites in feces and urine has also rarely been reported. The aim of this article is to summarize the current state of knowledge on the hormonal control of pregnancy and parturition in SAC and OWC and based on this, to show the possibilities for hormonal pregnancy diagnostics. Essential prerequisites for broader application, particularly of non-invasive methods in routine diagnostics, are the optimization of previously pursued methodological approaches, the commercial availability of the necessary reagents at reasonable cost, and the establishment of reliable reference values.

Keywords: estrogens, Old World camels, pregnancy-associated glycoproteins, progesterone, relaxin, South American camelids

Introduction

Camelidae are classified as Tylopoda within the Order Artiodactyla (even-toed ungulates). The extant members of the Camelidae family are divided into the groups of Old World camels (OWC, Tribe: Camelini) and South American camelids (SAC, Tribe: Lamini). Currently, two extant OWC species are described: the dromedary (*Camelus dromedarius*) and the Bactrian camel (*Camelus bactrianus*) (Wheeler 1995). However, the wild form of the Bactrian camel (*Camelus bactrianus ferus*), which occurs in northwest China and Mongolia and is critically endangered, is sometimes also considered a separate species (Ji et al. 2009). The SAC include a total of four extant species: the guanaco (*Lama guanicoe*), the vicuña (*Vicugna vicugna*), the llama (*Lama glama*), and the alpaca (*Vicugna pacos*) (Kadwell et al. 2001). Guanaco and vicuña are wild forms from which the corresponding domesticated forms, llama and alpaca, have evolved. Accordingly, llama and guanaco are assigned to the genus *Lama*, while alpaca and vicugna form the genus *Vicugna*. The species concept is sometimes questioned for OWC and SAC, as crosses between species of either group produce fertile hybrids. Nonetheless, for simplicity, this article refers to the different OWCs and SACs as species.

It is estimated that around 35,000 SACs are currently kept in Germany. The population of OWC is estimated to be significantly smaller and there are currently only a few hundred animals (oral information from the association "Altweltkamele e.V.") in Germany. SAC are mainly kept as hobby animals, but farms exist that keep and breed these animals as a secondary or primary livelihood. The main uses include animal-assisted activities such as trekking, wool production, and breeding (Wagner et al. 2022). The keeping of OWC is primarily limited to zoos and wildlife parks, but an increasing number of private individuals and farms keep and breed these animals as well. As a result, there has been an increasing need for veterinary care for both OWC and SAC in recent years, especially in breeding farms, where specific knowledge of the reproductive biology of these animals is required. Compared to other domesticated ungulates, the reproductive biology and endocrinology of camelids show some significant peculiarities, and there are still considerable gaps in knowledge in certain areas. For example, there is relatively little confirmed information available on the hormone production related to pregnancy, as well as on the endocrine control of pregnancy and parturition. The aim of this work is therefore to summarize the current state of knowledge regarding the endocrinology of pregnancy and parturition, as well as the possibilities of hormonal pregnancy diagnosis in camelids.

The lineages OWC and SAC separated approximately 11-25 million years ago (Cui et al. 2007) and have since inhabited very different habitats and ecosystems, leading to respective adaptations. Nevertheless, studies on reproductive biology have shown many similarities between OWC and SAC. However, since there exist significant differences in reproductive endocrinology even among phylogenetically closely related species, comparative aspects will also be considered here.

Establishment of pregnancy and maternal recognition of pregnancy ("luteal rescue")

All camelids have induced ovulation, triggered by a signaling molecule from the seminal plasma (seminal plasma factor, ovulation inducing factor, OIF). In OWC and SAC, β-Nerve Growth Factor (β-NGF) has been identified as the signaling molecule underlying OIF (Ainani et al. 2022, Paiva et al. 2022). β-NGF is absorbed through the endometrium, transported via systemic circulation to the brain, and ultimately stimulates the anterior pituitary to release a pre-ovulatory LH peak. The information on the period between copulation and ovulation of the dominant follicle varies between 1 and 2 days, depending on the species and author (Table 1). If ovulation results in establishing a pregnancy, the resulting corpus luteum (CL) remains active throughout the entire gestation period, which is approximately 11-12 months in SAC and about 13-14 months in OWC. If ovulation does not lead to conception, a CL of pseudopregnancy forms but regresses after 1-2 weeks (Table 1). Ovulations occur roughly equally in both ovaries. If ovulation occurs on the right ovary, the conceptus must migrate to the contralateral uterine horn to maintain pregnancy, to suppress the release of luteolytic prostaglandin F2α (PGF2α). This is due to different luteolytic capabilities of the uterine horns. In addition to the local luteolytic effect that both uterine horns may exert on the ipsilateral ovary, the left uterine horn also has a luteolytic effect on the right ovary due to its special vascular architecture (Fernandez-Baca et al. 1979). These features lead to implantation of the conceptus in the left uterine horn in 98-100% of cases. Accordingly, pregnancies in the right uterine horn are possible but extremely rare (Sumar 1999).

A fundamental requirement for establishing and maintaining pregnancy across species is sufficient progestogen supply, with progesterone (P4) being the dominant hormone with few exceptions. In early pregnancy, P4 is provided by the CL irrespective of the species (see below). In many mammalian species,

Table 1. Information on formation of a corpus luteum (CL) and luteal regression after unsuccessful mating in Old World camels and South American camelids. The lifespan of a CL of pregnancy practically corresponds to duration of pregnancy, which is also listed here. D, h: Days, hours after mating.

	Alpaca	Llama	Dromedary	Bactrian camel
Time between mating and ovulation	26 h (San-Martin et al. 1968); 30-72 h (Sumar et al. 1993)	30±0.5 h (Ratto et al. 2006); D1.8 (Adams et al. 1989); D1.9-2.1 (Adams et al. 1990)	28-36 h (Skidmore 2011)	36-48 h (Xu et al. 1985)
Increase in P4 concentrations after mating	from D4 (Aba et al. 1995); from D5 (Sumar et al. 1988)	starting between D3-6 (Ratto et al. 2006); from D4 (Adams et al. 1991, Aba et al. 1995); from D5 (Sumar et al. 1988)	from D3 (Skidmore et al. 1996); from D4-D5 (Marie und Anouassi 1987)	from D5 (Xu et al. 1985)
Time span of maximum P4 concentrations in the case of unsuccessful mating	D7-8 (Sumar et al. 1988)	D7-8 (Sumar et al. 1988); around D9 (Ratto et al. 2006)	D7-11 (Marie und Anouassi 1987)	n/a
Occurrence of PGF2α peaks or increased PGF2α concentrations after unsuccessful mating	D9-11 (Aba et al. 1995); D9-13 (Sumar et al. 1988)	D9-11 (Aba et al. 1995); D9-13 (Sumar et al. 1988)	D10 (Skidmore et al. 1998)	n/a
Loss of CL function after unsuccessful mating	D10-11 (Aba et al. 1995); D12 (Sumar et al. 1988); D8-13 (Fernandez-Baca et al. 1970)	D10-11 (Aba et al. 1995, Aba et al. 2000); D12 (Sumar et al. 1988)	D10-11 (Skidmore 2011; Skidmore et al. 1998); D11-14 (Marie und Anouassi 1987)	n/a
Frequency of spontaneous ovulation	5% (Fernandez-Baca et al. 1970); 4-10% (Smith et al. 1994)	8% (Adams et al. 1990)	non-lactating animals; 5% of animals (1.4% of follicular waves); lactating animals: 41.7% of animals (14.3% of follicular waves) (Nagy et al. 2005)	n/a
Duration of gestation (days)	342-345 (San-Martin et al. 1968)	344 (range: 331-347) (Johnson 1989); 345±8 (range: 327-357); no difference between pregnancies with a male or female calf (Sumar 1999)	384.3±10.9 (range: 344-420) (Bene et al. 2021); 384.5±0.9 (range: 333-422); difference between pregnancies with a male vs. female calf is statistically significant but minimal (Nagy and Juhasz 2019)	402.2±11.5 (Chen and Yuen 1984)

n/a – no relevant information to be found

especially poly-estrous ones, the lifespan of CL in the absence of pregnancy is significantly shorter than the gestation period. To avoid premature termination of pregnancy, the premature regression of the CL must be prevented by a specific early embryonic signal ("maternal recognition of pregnancy" (MRP) or "luteal rescue"). Over the course of evolution, different MRP signals and mechanisms have developed in a species-specific manner, many of which are still unknown. Examples include interferon τ (ruminants), chorionic gonadotropin (primates), prolactin/placental lactogen (mouse, rat) and trophoblast estrogens (pig) (Braz et al. 2024).

In camelids, after ovulation, a CL forms, which from day 4-5 after mating results in a measurable increase in P4 concentration in the systemic maternal circulation. In pregnant and non-pregnant animals, maximum P4 levels are reached between days 7-9 (see Table 1). After an unsuccessful mating, basal concentrations are already measured again after 1-2 weeks. During the regression of a CL of pseudopregnancy (OWC: days 10-11 after mating; SAC: days 8-13 after mating), increased concentrations of PGF2α of endometrial origin are observed in the systemic blood of non-pregnant camelids, similar to the cyclic luteolysis seen in cattle, pigs, and horses (usually measured as concentrations of the stable PGF2α metabolite 15-keto-13,14-dihydro-PGF2α, PGFM). In contrast to cattle, pigs, horses and domesticated SAC (Sumar et al. 1988), elevated concentrations but no pronounced PGF2α peaks were detected in dromedaries despite frequent sampling (Skidmore et al. 1998). In pregnant

Table 2. Information on concentrations of pregnancy-associated estrogens in maternal blood in Old World camels and South American camelids.

Estrogen	Feature	Alpaca	Llama	Dromedary	Bactrian camel
Estrone sulfate	beginning or duration of prepartal increase	last three months of gestation (Aba et al. 1998); from D330 (Bravo et al. 1996b)	last three months of gestation (Aba et al. 1998); from D330 (Bravo et al. 1996b)	from D330 (Skidmore et al. 1996)	n/a
	average maximum concentrations	42 ng/ml (Bravo et al. 1996b); 18±5 nmol/l (Aba et al. 1998); 3.4±0.6 ng/ml (Volkery et al. 2012)	42 ng/ml (Bravo et al. 1996b); 15±3 nmol/l (Aba et al. 1998)	46±3.8 ng/ml (Skidmore et al. 1996)	n/a
	decline at the end of gestation	on the day after parturition (Aba et al. 1998)	on the day of parturition (Aba et al. 1998)	two days prior to parturition (Skidmore et al. 1996)	n/a
Estradiol-17β	beginning or duration of prepartal increase	last 45 days prior to parturition (Aba et al. 1998)	last 45 days prior to parturition (Aba et al. 1998)	from D300 (Elias et al. 1984, Skidmore et al. 1996); from the 5th month of gestation (Agarwal et al. 1987)	from D300 (Zhao et al. 1998)
	average maximum concentrations	180 pmol/l (Aba et al. 1998)	180 pmol/l (Aba et al. 1998)	around 450 pg/ml (Agarwal et al. 1987); 518.7±2.2 pg/ml (Skidmore et al. 1996); 606±120 pg/ml (Elias et al. 1984)	1213 pg/ml (Zhao et al. 1998)
	decline at the end of gestation	on the day of parturition (Aba et al. 1998)	on the day of parturition (Aba et al. 1998)	on the day of parturition (Skidmore et al. 1996)	on the day of parturition (Zhao et al. 1998)

n/a – no relevant information to be found

llamas, the appearance of PGF2α pulses with comparable frequency has been described during the same period, but these pulses had significantly lower amplitudes than in non-pregnant animals. Consequently, the total amount of PGF2α released is only about 3% of the amount released during luteolysis. Since this amount is insufficient to trigger luteolysis, pregnancy remains intact (Aba et al. 1997, 2000).

To prevent luteolysis, an MRP signal must have a sufficient effect early enough before it begins. In OWC and SAC, it is therefore assumed that the effect of the MRP signal must begin before day 10-12 of pregnancy (Skidmore et al. 1998, Bianchi et al. 2023). The nature and mechanism of action of the MRP signal in camelids are not yet fully understood. Based on observations that, in llamas, the blastocyst produces significant amounts of estradiol-17β (E2) from day 7 after mating, it has been postulated that, similar to pigs (Bazer and Thatcher 1977), estrogens derived from the trophoblast serve as the MRP signal (Bianchi et al. 2023, Braz et al. 2024). Accordingly, in dromedaries, high estrogen production has been detected in embryonic tissue fragments between days 10 and 33 after ovulation (Skidmore et al. 1994). The hypothesis that trophoblastic estrogens act as the MRP signal in camelids is further supported by an experiment in llamas, where ovulation was induced via human chorionic gonadotropin (hCG), followed by the intramuscular administration of a long-acting E2 preparation between days 7-15 afterward. High doses of E2 resulted in significantly increased P4 production and delayed regression of the CL compared to llamas receiving a lower E2 dose or placebo (Powell et al. 2007). The generally accepted concept in pigs that trophoblast estrogens are the MRP signal in this species is largely based on a similar experimental approach. However, in non-pregnant pigs, administration of E2 depending on the experimental conditions has induced a prolongation of the cyclic luteal phase to over 60 days (Geisert et al. 1987), whereas in llamas, luteolysis was only delayed by a few days. Consistent with a role of trophoblast estrogens as the MRP signal, Bravo et al. (1996b) measured short-term increases in estrone sulfate (E1S) in the blood and urine during early pregnancy in llamas and alpacas. In the blood, the E1S increase began on day 21, reached peak levels on day 23 – comparable to those in late pregnancy – and returned to near basal levels by day 25. A significant increase in E1S concentration was also observed in the urine between days 18-27. However, the sharp, high estrogen peak in blood plasma must be

viewed with some skepticism, as no other comparable observations during early pregnancy in SAC have been reported so far, and the concentrations published in this study during late pregnancy differ significantly from other studies (Aba et al. 1998, Riveros et al. 2009, Volkery et al. 2012). In dromedaries, two moderately pronounced increases in E1S concentrations in blood plasma during early pregnancy have been described, with maximum values around day 25 and day 75, but these are considerably lower than the peak concentrations observed in the final stages of pregnancy (Skidmore et al. 1996) (see Table 2). No relevant information was found in the literature regarding the Bactrian camel.

Profile and sources of progesterone during pregnancy

Regardless of the species, the CL is the relevant source of P4 during early pregnancy. As pregnancy progresses, the primary source of progestogens varies significantly depending on the endocrine function of the placenta in each species (Schuler 2023a). In some species, such as dogs, no placental steroid hormone production has been detected, and the ovaries remain the sole significant source of P4 throughout pregnancy. In other species, the placenta takes over the role of the main source of pregnancy-sustaining progestogens after a certain proportion of the gestation period, and the ovaries no longer play a role in the production of progestogens in advanced stages of pregnancy ("luteo-placental shift"; examples include sheep, horses, guinea pigs and humans). In several animal species, the ovaries are the only quantitatively significant source of P4 throughout pregnancy, although placental P4 production is qualitatively detectable (examples: goat, pig). The specific significance of placental P4 in these cases remains unclear due to its negligible contribution to maternal blood levels; it may have local functions in the fetal-maternal contact zone or might merely be a byproduct or intermediate product of placental estrogen production (Schuler et al. 2018, Schuler 2023a).

In the case of OWC and SAC, the administration of luteolytic prostaglandins consistently results in a prompt abortion, regardless of the stage of pregnancy (Sumar 1988, Smith et al. 2000). This suggests that luteal P4 is essential throughout pregnancy in camelids, and the placenta contributes only minimally, if at all, to maternal P4 levels. Consistent with this, well-developed corpora lutea were always detectable in pregnant dromedaries between day 60 of pregnancy and until the end of the gestation period (Elwishy et al. 1981). The ability of the placentae of OWC and SAC to synthesize P4 is supported by the fact that they produce significant amounts of estrogen and thus express also the necessary enzyme systems for P4 synthesis (CYP11A1, HSD3B; Wooding et al. 2003).

For OWC and SAC, P4 concentrations remained largely constant throughout pregnancy. In some cases, moderately elevated levels were reported during early pregnancy (dromedary: Agarwal et al. 1987, Skidmore et al. 1996) while a gradual decrease was observed in the last month of pregnancy before the steep prepartum decline in P4 (dromedary: Elias et al. 1984, Skidmore et al. 1996; guanaco: Riveros et al. 2009; llama: Leon et al. 1990, Aba et al. 1998).

Placental endocrine function

The placenta serves as an essential interface between the maternal uterus and the developing fetus and has several important functions during pregnancy. Over the course of evolution, an intriguing diversity of placental structures has developed among mammals. The placentae of camelids are classified in terms of their structure as epitheliochorial, diffuse and villous (Wildman et al. 2006, Carter 2024). In addition to numerous other tasks, the placenta also functions as a temporary endocrine organ. Besides hormones in the classical sense – i.e., regulatory factors that reach their target cells via the systemic circulation – signaling molecules with local effects in the fetal-maternal contact zone are primarily produced in the placenta (e.g., para-, auto-, or intracrine factors). Placental signaling molecules are involved in regulating a wide variety of processes in the mother and fetus, and especially within the placenta itself. Among the structures involved in the development of the placenta, the chorionic epithelium (trophoblast) is a particularly rich source of various regulatory factors. During evolution, not only has an extraordinary variety of placental structures developed in mammals, but the "placental endocrine function" also exhibits considerable species differences, which can even occur between phylogenetically closely related species (Schuler et al. 2018, Schuler 2023a, 2023b). Unlike many other domestic mammal species, there is currently relatively little information available regarding the placental endocrine function in camelids.

Placental estrogen synthesis

In many, but not all, mammalian species, the production of steroid hormones in the placenta has been demonstrated. Besides P4 or other pregnancy-maintaining progestogens, a particularly high production of estrogens is detectable in the placentas of primates and ungulates, primarily occurring in the chorionic epithelium (trophoblast). Regarding the spectrum of estrogens

circulating in maternal blood, the concentration profile over the course of pregnancy and the synthesis pathways involved, significant species differences can be observed (Schuler 2021, 2023b). Many questions about the significance of placental estrogens remain open. Generally, in late gestation, they are associated with the preparation of the myometrium and birth canal for delivery and preparation of the mammary gland for the onset of lactation. Since placental estrogen production is detectable locally even in early pregnancy stages, various additional functions in the mother, fetus, and placenta itself are postulated depending on the species; stimulation of angiogenesis in the fetal-maternal contact zone, regulation of uteroplacental blood flow, promotion of uterine growth, and control of placental growth and differentiation may be such functions (Schuler et al. 2018). In some species, significant estrogen production by trophoblasts has been demonstrated as early as the blastocyst stage, including horses, pigs, llamas and dromedaries (see above).

Information on estrogen profiles throughout pregnancy is very limited for camelids. However, existing data clearly indicate that, like domestic ruminants and equids, conjugated estrogens dominate in the maternal blood of pregnant OWC and SAC, far exceeding free estrogens, with E1S likely being the main component. Specific information on concentrations and pregnancy profiles for different Camelidae species is provided in Table 2. The fact that E1S is an inactive estrogen metabolite suggests that the primary site of action for placental estrogens is in the pregnant uterus (Schuler et al. 2018, Schuler 2021). Unlike equids, which exhibit a pronounced concentration maximum around mid-pregnancy, camelids – like domestic ruminants – show a pronounced increase in placental estrogens only in the last trimester of pregnancy, with peak concentrations measured in the last month of gestation. In some cases, the pregnancy profiles and peak concentrations reported for SAC differ considerably, whereby these differences can only partly be explained by variations in the measurement methods, such as different sensitivities of the measurement methods or different cross-reactivities of the antibodies used in the immunoassays. For example, the final rise in E1S concentrations, according to Aba et al. (1998), begins in maternal blood approximately three months before birth in alpacas and llamas. In contrast, Bravo et al. (1996b) reported that in llamas and alpacas, the steep prepartum increase in E1S occurs only in the last month of pregnancy, with peak concentrations about ten times higher than those published by Aba et al. (1998). In dromedaries, a clear increase in E1S concentrations begins after the 300th day of pregnancy. Peak concentrations are reached about 2-4 weeks before birth and are significantly higher than in SAC. There are no corresponding data found in the literature for Bactrian camels. For E2, similar profiles to E1S are described in maternal blood, but its concentrations are comparatively minimal. More detailed information on pregnancy-specific estrogen production in camelids is available for the dromedary and, to a lesser extent, for the llama and alpaca. In the dromedary, the enzymes necessary for estrogen synthesis (CYP11A1, HSD3B, CYP17A1, CYP19A1) were detected exclusively in mononuclear trophoblast cells between days 14-25 post-ovulation. With the appearance of trophoblast giant cells around day 30 post-ovulation, the expression of steroidogenic enzymes in mononuclear trophoblast cells disappears and is thereafter detectable only in trophoblast giant cells until the end of pregnancy. Similarly, in term placentas of alpacas and llamas, the aforementioned steroidogenic enzymes are found exclusively in trophoblast giant cells. It is currently unclear whether the steep prepartum increase in estrogens is due to an increase in activity or a higher number of estrogen-producing trophoblast giant cells (Wooding et al. 2003).

Pregnancy-specific relaxin production in camelids

Relaxin is a peptide hormone from the insulin-like gene superfamily, which includes insulin, insulin-like growth factors -1 and -2 and the insulin-like peptides (INSL)-3, -4, -5 and -6 in addition to the relaxin paralogs. Relaxin 1 (RLN; relaxin 2 in higher primates), which is significantly associated with reproductive functions, was discovered around 100 years ago due to its softening effect on the pelvic symphysis in guinea pigs (Hisaw 1926). Since then, depending on the species, RLN expression has been described in various tissues within and outside the female and male reproductive systems, and a significant number of functions have been identified. In several species, a clear increase in maternal RLN levels has been observed during pregnancy, which could primarily be attributed to the ovary (e.g., pig, human) or the chorionic epithelium of the placenta (e.g., dog, horse), depending on the species. Examples of pregnancy-associated functions of RLN include, in a species-dependent manner, the loosening of the pubic symphysis, promotion of endometrial angiogenesis, stimulation of mammary gland nipple growth, modulation of myometrial activity, stimulation of uterine growth, and cervical ripening. Ruminants do not express RLN as a special feature. In their genomes, only an inactive pseudogene (e.g., sheep) is detectable, or the relaxin 1 gene has apparently been completely lost during evolution (e.g., cattle) (Bathgate et al. 2013, Malone et al. 2017).

In contrast to ruminants, OWC and SAC do express

RLN. The preproRLN mRNA of the dromedary has been fully cloned (Hombach-Klonisch et al. 2000; NIH National Library of Medicine sequence ID: AF254739). Corresponding, nearly identical nucleotide sequences are also "predicted" for the Bactrian camel (both domesticated and wild forms; sequence IDs: XM_074361611.1, XM_032477726.1) and for the alpaca (XM_006208144.4). Information on RLN expression in the ovaries and the uteroplacental compartment during pregnancy is currently available only for the dromedary, where the strongest signals at the mRNA and protein levels (in-situ hybridization, immunohistochemistry with primary antibodies against pig RLN) were detected in luteal cells and the luminal uterine epithelium (Hombach-Klonisch et al. 2000, Osman et al. 2000). In llamas and alpacas, a significant increase in maternal RLN blood levels was observed during pregnancy in the third month, which persisted until birth (Bravo et al. 1996b, Volkery et al. 2012). In these studies, immunoassays specific to porcine RLN were used. Currently, there is no definitive information on the significance of pregnancy-specific RLN production or the origin of RLN circulating in maternal blood in camelids.

Pregnancy-associated glycoproteins (PAGs)

PAGs represent a group of molecules that are specifically expressed during pregnancy in Cetartiodactyla (even-toed ungulates and whales), initially in the pre-placental trophoblasts and, with the onset of placentation, in the placental trophoblast. They belong to the gene superfamily of aspartic proteases. Other members of this superfamily include digestive enzymes such as pepsin and chymosin, as well as renin. PAGs and the other aspartic proteases apparently originate from gene duplications of a common ancestral gene. In the genomes of Cetartiodactyla species, individual PAG genes have been identified that arose through gene duplications approximately 87 million years ago and are referred to as "ancient PAGs". Around 52 million years ago, further PAG genes ("modern PAGs") emerged in ruminants through additional gene duplications, with a particularly high number found in Bovidae (e.g., cattle, sheep, goat). Accordingly, a larger number of PAG genes have been identified in ruminant species, whereas only a few PAG genes have been detected in other Cetartiodactyla species so far. Despite years of intensive research – especially in cattle – the biological significance of PAGs remains largely unclear. Due to their common origin with proteases, some PAGs exhibit proteolytic activity. For others, this ability appears to have been lost during evolution, although the capacity for protein binding may have been retained. Depending on the species and the specific PAG molecule, various functions have been postulated, such as modulation of the maternal immune system, activation of signaling molecules through proteolytic cleavage, luteotropic effects via interaction with maternal LH receptors, or influencing cell adhesion at the fetal-maternal interface. However, a definitive confirmation of these functions has not yet been achieved (Hughes et al. 2003, Wallace et al. 2015). In OWC and SAC, there is currently very limited specific information regarding the presence of PAG genes, the properties of their gene products, and their functions. Using reagents specific to cattle or pigs, positive reactions have been observed at the mRNA and protein levels in placental tissues of OWC and SAC (Bella et al. 2007, Majewska et al. 2009, 2011, 2013). Therefore, it is assumed that like other Cetartiodactyla species, camelids also possess and express PAG genes.

In ruminants, some PAGs are released in significant quantities through the placental barrier into the maternal compartment and can be detected in maternal blood and milk from early stages of pregnancy. Methods for measuring PAGs in blood and milk have been developed for cattle and other ruminants and can be used for reliable pregnancy diagnosis (Barbato et al. 2022). It is currently unknown to what extent PAGs occur in the maternal blood of OWC and SAC and whether they could serve as parameters in hormonal pregnancy diagnostics.

Endocrine control of parturition

Currently, there is limited confirmed information about the signaling cascade that leads to the initiation of birth in camelids. Studies on hormone levels in systemic maternal blood during the immediate prepartal period have yielded results largely consistent with those known from other domestic artiodactyls. The immediate prepartum decline in P4, an increase in estrogens, and a massive rise in PGFM in the last hours before expulsion of the fetus are such findings. This substantial prepartal increase in PGFM in systemic maternal blood appears to be primarily associated with the activation of the myometrium (Leon et al. 1990, El-Belely 1994, Aba et al. 1998, Schuler et al. 2018, Schuler 2023a, 2023b). As mentioned earlier, previous observations suggest that in both OWC and SAC, the ovaries remain the only significant source of P4 until the end of pregnancy. Therefore, it is assumed that prepartum luteolysis is a crucial prerequisite for a physiological onset of labor in camelids. Consistent with this concept, parturition in late-pregnant alpacas can be reliably and promptly induced by administering a prostaglandin F2α analog (40 µg fluprostenol i.m.; Bravo et al. 1996a). In rumi-

nants, a sharp prepartum increase in fetal cortisol has been demonstrated to play a central role in the signaling cascade leading to birth. Consistently, in these species birth can be effectively induced by glucocorticoid administration (Schuler 2023b). However, for alpacas dexamethasone has proven unsuitable in this regard (Bravo et al. 1996a). A single intramuscular dose of up to 0.05 mg did not significantly shorten pregnancy duration. The administration of 0.5 mg dexamethasone – a dose far below the 10-20 mg commonly used in sheep and goats - resulted in stillbirths after 6-9 days in all cases, with the circumstances indicating that the death of the placenta and fetuses occurred soon after dexamethasone administration. Despite the limited data, current information indicates significant differences between camelids and other domesticated ungulate species regarding the mechanisms controlling the onset of parturition (Schuler 2023a, 2023b).

Hormonal pregnancy diagnosis

Pregnancy diagnosis plays an important role in breeding management. After mating or insemination, early identification of non-pregnant animals is crucial for planning future breeding measures. In advanced stages, the focus is on ruling out pregnancy loss and confirming the integrity of the pregnancy. In OWC and SAC, especially due to the relatively high frequency of pregnancy losses in early pregnancy stages, a single pregnancy check is considered insufficient. After a positive early pregnancy diagnosis, a follow-up examination around the 3rd to 4th month is recommended (Skidmore 2000). Generally, clinical methods are preferred for pregnancy diagnosis because they provide direct evidence, often more comprehensive information, and results are available immediately. As with other domestic mammals, an accurate clinical diagnosis of pregnancy is also possible in camelids with the aid of sonography, but this can involve considerable effort in capturing and restraining the animals and carries a risk of injury for the animal and the examiner (Brown 2000, Runcan and Coutinho da Silva 2022). In particular, handling untrained OWC can pose considerable risks for staff. Furthermore, animal owners fear that the stress of handling could lead to a loss of pregnancy.

As an alternative to clinical diagnosis, hormone measurements in blood can also be used for pregnancy detection in camelids. Blood sampling in SAC, which is usually performed by puncturing the jugular vein, requires some experience due to the special anatomical conditions in the neck area and the rough texture of the skin. In untrained OWC, blood collection is often not possible without sedation. In such cases, non-invasive pregnancy diagnostic methods (see below) are particularly advantageous.

General aspects of hormonal pregnancy diagnostics

Suitable parameters in hormonal pregnancy diagnostics are hormones that are only detectable in the event of pregnancy (e.g. chorionic gonadotrophin in horses) or whose concentrations in the event of pregnancy exceed the measured values of non-pregnant animals as much as possible on an individual level. Depending on the parameter, the applicability of a particular method is limited to a specific period of gestation. In camelids, depending on the parameter and species, experience is available from the measurement of P4, pregnancy-associated estrogens and RLN (see below). Despite the currently increasing availability of mass spectrometric measurement methods, particularly in steroid analysis, immunological measurement methods are still in the foreground, especially in routine endocrinological diagnostics. A particular difficulty in hormonal pregnancy diagnostics in camelids is that there are hardly any commercially available measurement methods that have been specifically developed and validated for use in this species group. In principle, measurement methods developed for other species can also be used successfully in camelids. In the case of proteo- or peptide hormones such as RLN, the prerequisite is a sufficient cross-reaction of the antibodies used with the corresponding target molecule of the camelids due to their species-specific structure. Although the structure of steroids is species-independent, matrix effects (species-specific interfering effects of sample components) can mean that a steroid assay can only be used with considerable restrictions or is completely unsuitable for certain species or sample materials. In any case, a measurement method must be adequately validated for the respective target species and also for the intended sample material before use (Wudy et al. 2018).

Special aspects of hormonal pregnancy diagnostics in camelids using blood samples

Since ovulation in camelids is usually triggered by mating, pregnancy diagnostics can be carried out with a high degree of certainty in these species by measuring P4 after the end of the lifespan of a CL of pseudopregnancy (see Table 1) over the entire remainder of the gestation period. With basal P4 values, pregnancy can be reliably ruled out. False positive diagnoses due to spontaneous ovulation or unobserved unsuccessful mating are possible in a small percentage of cases. Furthermore, the occurrence of long-lived persistent CL or luteinized follicles has been described in non-pregnant llamas (Adams et al. 1989), dromedaries (Almushawwah et al. 2025) and Bactrian camels

(Fowler and Santymire 2022), which can also lead to false positive results. P4 concentrations ≥ 2 ng/ml are often considered a positive finding regarding the presence of pregnancy. However, it should be borne in mind that the interpretation may have to be based on a method-specific limit value (see above) and that in the last month of pregnancy – also according to our own experience with SAC – the P4 concentration can be significantly below this limit even with intact gravidities (Bravo 1994). The detection of pregnancy-associated estrogens can also provide very reliable proof of pregnancy. As in other ungulate species, E1S, which circulates in the maternal blood in much higher concentrations than the free estrogens, E2 or estrone, is particularly suitable for this purpose. However, according to current data, the increase in placental estrogens in the blood of dromedaries and SAC can only be reliably detected in the last two months of pregnancy (Bravo et al. 1996b, Skidmore et al. 1996, Aba et al. 1998; see Table 2). In Bactrian camels, no conclusive data are yet available. It may also be possible to use a short-term increase in estrogen in early pregnancy to diagnose pregnancy in SAC (Bravo et al. 1996b; see above). At present, however, the data situation for this is still too weak. In llamas and alpacas, it has been shown that RLN determination can be used reliably for pregnancy diagnosis from the third month of pregnancy (Bravo et al. 1996b, Volkery et al. 2012). No study has yet been published on this in OWC.

Non-invasive hormonal pregnancy diagnostics in camelids

In uncooperative animals, especially in OWC, taking blood samples can also be difficult or impossible without sedation. In such cases, non-invasive hormone measurements are an attractive alternative. In SAC and probably also in OWC, the measurement of P4 or placental estrogens or their metabolites in milk, urine or feces may be suitable for this purpose.

The collection of milk samples from SAC is much more difficult than from domestic ruminants or horses, also according to our own experience, as the udder is visited and emptied by the crias at short intervals. Furthermore, milk sampling is obviously perceived as stress by a considerable proportion of the animals, to which they react by withholding milk. Therefore, sufficient volumes of cleanly collected sample material can often only be milked after a temporary prevention of suckling, if necessary, after application of oxytocin (Riek and Gerken 2006, Mößler et al. 2021). Furthermore, milk is no longer available as sample material in camelids after the end of lactation over a longer period of pregnancy. Volkery et al. (2012) found a highly significant difference between the P4 concentrations in the milk of pregnant and non-pregnant alpacas and therefore considered milk P4 measurement to be a suitable method for non-invasive pregnancy diagnostics. There is also experience with the determination of P4 in milk from dromedaries. These indicate that the milk P4 test can also be used for pregnancy diagnosis in this species (Abdel Rahim and el-Nazier 1987).

Depending on the specific reproductive endocrinology of the target species, sex steroids can also be measured non-invasively in feces for pregnancy diagnostics. However, in general the ease of sample collection is offset by significantly higher analytical requirements. Before steroid determination, fecal samples must be processed using suitable extraction procedures to eliminate matrix effects before the actual measurement (for methodological details see Palme 2005). Steroids are excreted into the intestine mainly via the bile after they have been metabolized in the liver depending on the respective molecule and species. In bile, steroids are predominantly present in conjugated form, i.e. coupled to sulfate or glucuronic acid. In feces, steroids are exposed to the metabolic activity of intestinal bacteria. Due to the hydrolytic activity of intestinal bacteria, conjugated steroids are mainly converted back into free forms and can undergo further transformations. Some of the steroids are reabsorbed from the intestine and, depending on the molecule, are excreted again in the feces (enterohepatic circulation) or urine. Therefore, the original hormone often appears in feces in low concentrations at best. Instead, the excretion of sex steroids via the feces occurs predominantly in the form of a species-specific metabolite spectrum. Therefore, special measurement methods adapted to the target species and the sample material usually must be used for steroid analysis in feces. In addition to specific assays for one of the main metabolites (if known), antibodies with cross-reactivity against a wide range of possible metabolites ("group-specific antibodies") are particularly advantageous (Schwarzenberger et al. 1996). In general, immunoassays developed for the determination of the original steroid hormone are only useful for pregnancy testing with fecal samples if the antibodies used cross-react to a sufficient extent with quantitatively significant metabolites. Information on pregnancy diagnostics in camelids based on steroid determinations in feces is only available to a very limited extent. Fowler and Santymire (2022) published pregnancy profiles for fecal P4 and estrogen metabolites (FPM, FEM) in two gravid Bactrian camels. Due to the small number of animals examined, the informative value of the results obtained is considerably limited from the outset. During pregnancy, the FPM concentrations were on average 2.9 times higher than the individual baseline of

the animals, although at individual time points they were below the baseline. Thus, the method used in this study to measure FPM can only be considered suitable for non-invasive pregnancy diagnostics in Bactrian camels with substantial restrictions. The FEM concentrations in both animals were consistently above the defined baseline in the last trimester. In early and mid-pregnancy, however, they fell below the baseline in some cases. As in blood and urine, pregnancy diagnostics based on the detection of pregnancy-specific estrogens in feces is presumably also limited to the final phase of pregnancy.

Egi et al. (2024) determined the fecal P4 concentrations in alpacas in the period between mating and day 60 p.c. using a commercial P4-specific ELISA. After day 15 p.c., there was no longer any overlap between the measurements for pregnant and non-pregnant animals. However, as the reference ranges for pregnant and non-pregnant animals were immediately adjacent to each other, confirmation of the diagnosis by sonography was recommended for measurements in the lower reference range for pregnant animals. Marozzi et al. (2024) published pregnancy profiles for FEM and pregnanediol glucuronide (PdG) in the feces of guanacos, whereby the antiserum used in the PdG assay showed a high cross-reactivity with other P4 metabolites including free steroids. The FEM concentrations showed a similar course to the E1S concentrations previously reported for blood (Riveros et al. 2009) with a clear increase only in the late phase of pregnancy, which can be used for reliable pregnancy diagnostics in the last two months of gestation. As with blood, in guanacos pregnancy diagnostics based on the determination of FEM is therefore presumably limited to the final phase of pregnancy. The extent to which the slight pregnancy-specific increase in "PdG concentrations" can be used for pregnancy diagnostics is not clear from this publication. Schwarzenberger et al. (1995) determined FPM in the feces of wild, temporarily captive vicuñas using two different immunoassays, whereby the group-specific antisera used were directed against P4 metabolites with a 20-oxo or a 20α-hydroxy group. Immunograms after HPLC separation revealed the presence of several unconjugated 5α- or 5β-reduced P4 metabolites. The authors concluded from their results that the measurement of FPM enables non-invasive detection of CL function in vicuñas. An evaluation of the methods used regarding their reliability in pregnancy diagnostics was not possible under the conditions of this study, as there was no confirmation of pregnancy by an independent method and the animals were released back into the wild before birth.

The excretion of sex steroids in urine also occurs predominantly in metabolized form. PdG was identified as the main metabolite of P4 in urine in various species. Accordingly, Volkery et al. (2012) found a highly significant difference in urinary PdG concentrations between pregnant and non-pregnant alpacas. This suggests that PdG measurement in urine could be used for hormonal pregnancy diagnostics such as P4 measurement in blood. However, as the antiserum used in the measurements by Volkery et al. (2012) showed a high cross-reactivity with several other P4 metabolites, it is unclear which P4 metabolites the measured signal was based on.

Pregnancy-associated estrogens are predominantly excreted in the urine of ungulates in conjugated form. In urine samples from alpacas and llamas, a diagnostically useful difference in concentration for E1S was only found, as in blood, in the final phase of pregnancy (Bravo et al. 1991, 1996b, Volkery et al. 2012). RLN was not measurable in the urine of alpacas (Volkery et al. 2012). The concentrations of hormones or their metabolites in urine can be influenced to a considerable extent by urine concentration. Therefore, their concentrations in urine are usually related to the concentration of creatinine, which is excreted in urine in largely constant amounts (Bravo et al. 1991, 1996b, Volkery et al. 2012).

Steroid measurements can also be carried out non-invasively in saliva, whereby the measurement of salivary cortisol in animals is used in particular for the non-invasive recording of stress states. The concentrations in saliva are considerably lower than in blood (Cobb et al. 2016), which is why the measurement methods used must have a high sensitivity. Volkery et al. (2012) determined the concentrations of P4, PdG and E1S in saliva samples from alpacas and were unable to determine diagnostically useful differences between pregnant and non-pregnant animals for any of the parameters mentioned. The RLN concentrations in saliva also did not differ between pregnant and non-pregnant alpacas (Volkery et al. 2012). Studies on non-invasive pregnancy diagnostics based on hormone measurements in the saliva of other SAC or in OWC are currently not available.

Conclusions

Compared to many other domestic animal species, relatively little information is available on the endocrine control of pregnancy and birth in camelids. However, some of the information available to date points to considerable differences to other domestic ungulate species. Knowledge of pregnancy-associated hormonal changes forms the basis for hormonal pregnancy diagnostics. Although clinical pregnancy diag-

nostics using sonography is of primary importance, hormonal methods, especially non-invasive methods, are potentially significant because they enable low-stress or stress-free sample collection. Non-invasive methods of pregnancy testing are of particular interest in untrained OWC, where clinical diagnostics or blood sampling without sedation can be associated with unacceptable risk to the personnel involved.

Apart from measuring P4 in blood (or to a lesser extent in milk), there is currently sporadic experience with relaxin (blood), pregnancy-specific estrogens (blood), and determining P4 and estrogen metabolites in fecal or urine samples. These isolated observations suggest that measuring relaxin in blood and P4 metabolites in feces or urine in particular could be useful methods for diagnosing pregnancy in camelids. Routine use of hormonal pregnancy diagnostics in camelids has so far been hampered mainly by the widespread lack of validated, commercially available, and economically attractive measurement methods for these species, as well as the lack of reliable reference values, which may be assay-specific. New possibilities for non-invasive pregnancy diagnostics in camelids could arise in the future through steroid metabolom analysis in feces and urine due to the increasing availability of mass spectrometric methods.

Dedication

The authors dedicate this article to Prof. Dr. Dr. h.c. mult. Bernd Hoffmann, Giessen, on the occasion of his 85th birthday.

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Author Declarations

Ethics approval

Ethical approval was not required as no experiments were conducted in connection with this work.

Use of generative artificial intelligence

Generative artificial intelligence tools were not used in the preparation of the manuscript.

Conflict of interest

The authors declare that they have no financial, personal, or institutional relationships that could be perceived as influencing the research.

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