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REVIEW

Advances in understanding canine pregnancy: Endocrine and morpho-functional regulation

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Abstract

Canine pregnancy relies on luteal steroidogenesis for progesterone (P4) production. The canine placenta responds to P4, depending on the nuclear P4 receptor (PGR). This has sparked interest in investigating the interaction between ovarian luteal steroids and the placenta in dogs. Canine placentation is characterized by restricted (shallow) trophoblast invasion, making the dog an interesting model for studying decidua-derived modulation of trophoblast invasion, compared with the more invasive (hemochorial) placentation. The PGR is expressed in maternally derived decidual cells and plays a crucial role in feto-maternal communication during pregnancy maintenance. Understanding PGR-mediated signalling has clinical implications for improving reproductive performance control in dogs. Altering the PGR signalling induces the release of PGF2α from the foetal trophoblast, hindering placental homeostasis, which can also be achieved with antigestagens like aglepristone. Consequently, luteolysis, both natural and antigestagen-induced, involves apoptosis, vascular lesion, and immune cell infiltration in the placenta, resulting in placentolysis and foetal membranes expulsion. Our laboratory developed the immortalized dog uterine stromal (DUS) cell line to study canine-specific decidualization. We study canine reproduction by observing physiological processes and investigating evidence-based mechanisms of decidualization and feto-maternal interaction. Our focus on morphology, function and molecular aspects enhances understanding and enables targeted and translational studies.

KEYWORDS
antigestagens, decidualization, dog (*Canis lupus familiaris*), feto-maternal communication, luteolysis, parturition, placenta, pregnancy

1 | PURPOSE OF THE PRESENT OVERVIEW

Recently, our research group has presented other comprehensive reviews that provided an overview of the current knowledge in the field. These reviews have functionally linked research results from our group and others, and helped to identify gaps to inform future research directions (Kowalewski et al., 2021; Kowalewski, Tavares Pereira, & Kazemian, 2020; Kowalewski, Tavares Pereira, Papa, et al., 2020). One of these reviews (Kowalewski, Tavares Pereira, & Kazemian, 2020) focused on the endocrine milieu and morpho-functional aspects of embryo- and feto-maternal communication during implantation, placentation and termination of pregnancy in the dog. The unique characteristics of canine placentation at

the morpho-macro and micro-functional levels were emphasized in (Kowalewski et al., 2021). Both articles highlighted the importance of decidualization in the underlying regulatory mechanisms. Finally, (Kowalewski, Tavares Pereira, Papa, et al., 2020) focused on exploring the mechanism of action and clinical and scientific use of antigestagens.

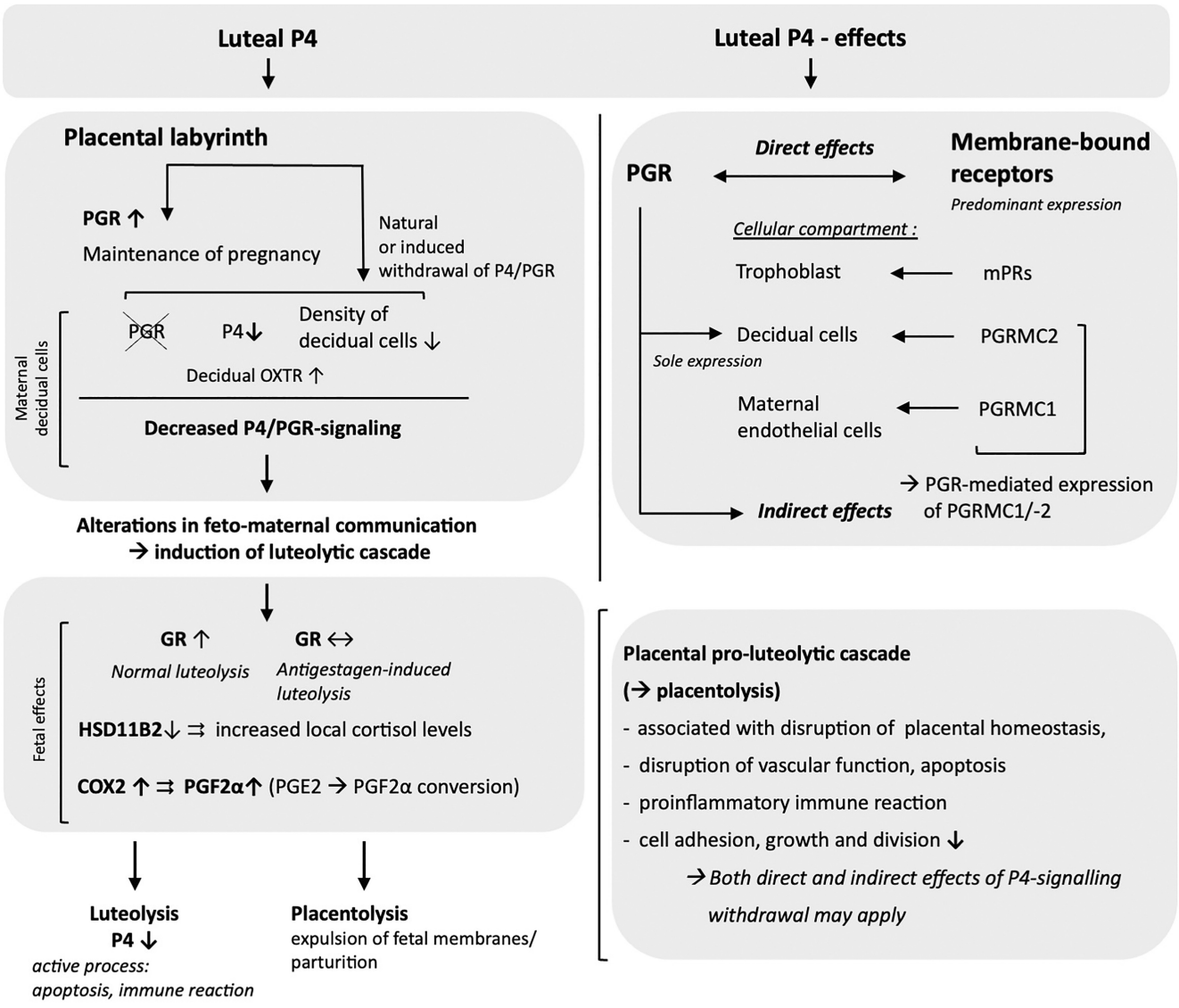
2 | ENDOCRINE PERSPECTIVE AND EMBRYO-MATERNAL INTERPLAY DURING PREPARTUM LUTEOLYSIS

The species-specific regulatory mechanisms and endocrine patterns observed in the dog strongly limit the possibilities of translational research. This includes the hormonal profiles and thus endocrine milieu associated with the establishment and maintenance of pregnancy.

As in other mammals, progesterone (P4) is needed for a successful outcome of canine pregnancy. Yet, unlike other domestic animal species, the dog lacks placental steroidogenesis, so canine pregnancy depends upon luteal provision of P4. Furthermore, there are similar secretion patterns of steroids in pregnant and non-pregnant (i.e. physiologically pseudopregnant) animals (for reviews see [Hoffmann et al., 2004; Kowalewski, 2014, 2017]). The canine placenta responds to circulating steroids by expressing progesterone receptors, including the nuclear PGR and, as shown in our most recent study, membrane-bound P4 receptors (Figure 1), as well as oestrogen receptors (ERα) and glucocorticoid receptors (GR/NR3C1) (Gram et al., 2016; Kazemian et al., 2023; Kowalewski et al., 2010; Vermeirsch, Simoens, Hellemans, et al., 2000; Vermeirsch, Simoens, & Lauwers, 2000). The canine placenta is also an endocrine organ, producing its own hormones, including relaxin (RLN), the only currently available marker of pregnancy in the dog produced by the foetal trophoblast (Nowak et al., 2017), and prostaglandins (Gram et al., 2013; Gram, Fox, et al., 2014; Kowalewski et al., 2010). Because dogs do not have a luteolytic response to the absence of pregnancy, they lack the classical recognition of pregnancy that is directed towards rescuing the corpora lutea (CL) from luteolysis (Kowalewski, 2014, 2017; Kowalewski et al., 2015; Zatta et al., 2017). Consequently, the species-specific maternal recognition of pregnancy appears to involve a coordinated interplay between the CL and the utero-placental compartment facilitating the onset and progression of pregnancy (Kowalewski et al., 2015). Implantation takes place on day 17–18 after fertilization and is followed by placentation shortly thereafter. Importantly, although the initiation of canine pregnancy occurs under conditions with high circulating P4 levels (30–35 ng/mL or even higher, reviewed in [Kowalewski, 2018]), exceeding those observed in other species, for example, large domestic animals, there is no induction of spontaneous decidualization despite the high degree of exposure of the uterus to this hormone (Graubner, Gram, et al., 2017). Following the gradually decreasing P4 levels, apparently associated with the luteal ageing and degenerative processes (Kowalewski, 2014; Papa & Kowalewski, 2020; Zatta et al., 2017), prepartum luteolysis appears to be activated at the placental level, where PGF2α is produced in the foetal compartment, in response to the fading luteal P4 synthesis and the hormonal levels reaching the lower threshold of approximately 2–3 ng/mL, associated with diminished P4-PGR signalling (Figure 1). Consequently, being characterized by a steep decline of circulating P4, luteolysis occurs 12–24 h before parturition (Concannon et al., 1989; Kowalewski et al., 2010, 2015), concomitant with the increased circulatory PGF2α originating from the trophoblast (Gram et al., 2013; Gram, Fox, et al., 2014; Kowalewski et al., 2010; Zatta et al., 2017). The nuclear PGR, which is localized solely in the decidual cells of the maternal placental compartment (Kowalewski et al., 2010; Vermeirsch, Simoens, Hellemans, et al., 2000), seems to play an important role in this regulatory process as the luteolytic cascade initiated in the placenta can be mimicked by the application of antigestagens (i.e. PGR blockers) (reviewed in [Kowalewski, 2014; Kowalewski et al., 2021; Kowalewski, Tavares Pereira, & Kazemian, 2020; Kowalewski, Tavares Pereira, Papa, et al., 2020]), which is also a feature used in our in vivo and in vitro studies.

As a further peculiarity of canine reproductive endocrinology, the circulating estradiol (E2) levels drop prepartum, as a result of both the lack of placental steroidogenesis and the prepartum luteolysis (Hoffmann et al., 1994). As shown in our studies, glucocorticoids appear to play a role at the placental level, perhaps being involved in the feto-maternal interplay during the termination of pregnancy (Gram et al., 2016; Tavares Pereira et al., 2023). The glucocorticoid receptor (GR/NR3C1) is localized in foetal trophoblast and is increased during the natural withdrawal of P4 (Gram et al., 2016; Figure 1). Yet, the circulating cortisol levels are very variable and are not indicative of the approach of parturition. Interestingly, the lack of an increase in the expression of *GR/NR3C1* following the antigestagen-mediated withdrawal of P4 indicates that it is not required for the initiation of PGF2α production (Gram et al., 2016) and other local roles of glucocorticoids may apply, as discussed previously (Gram et al., 2016; Tavares Pereira et al., 2023). Accordingly, in clinical settings, application of glucocorticoids does not appear to mimic the natural prepartum signalling cascade, as prolonged treatments are required for the termination of pregnancy and strong side effects occur (Wanke et al., 1997).

FIGURE 1 Schematic representation of proposed regulatory mechanisms involved in the regulation of canine placental function during pregnancy. The current understanding of the prepartum luteolytic cascade is presented in parallel with local luteal progesterone effects in different subcellular populations. The natural and/or induced withdrawal of placental PGR signalling leads to the activation of the placental pro-luteolytic cascade and placentolysis, suppression of luteal function, and the release of foetal membranes and, thus, parturition/abortion. The species-specific decidualization is embryo-induced, and leads to the development of decidual cells, with PGR withdrawal affecting their function.



Decidualization → decidual cells: **VS** **PGR withdrawal-driven effects in decidualized stromal cells and placenta:**

- embryo-induced
- cAMP-mediated (PGE2/other cAMP-inducers)
- involving reciprocal PGE2 ⇔ PGR interaction
- mesenchymal origin
  - → **mesenchymal-epithelial transition**
    - ⇉ retain VIM, aSMA, induce COL4↑, CX43↑, laminins
- decidualization markers↑: IGF1, PRLR, PGR, PTGES/EP4,
- decidual expression of: PGR, OXTR, ERα, RXFPR2, PGRMC1
- proliferation↑, cell adhesion↑ (e.g. SELP, PCNA, ANXA2)
- apoptosis↓
- kinases activity↑: ACG kinases, MAPK, *(…)*
- vasoactive and immunomodulatory effects: VEGFA, THSB2 *(…)*

→ modulation of trophoblast function
→ extracellular matrix and tissue remodelling
→ immunomodulatory effects (P4-mediated immunosuppression)

- upstream regulators: P4/PGR, dexamethasone, PPARγ, TGFβ
- ↓activity of over 80 kinases (including PKA)
- cell adhesion↓, proliferation↓, transcriptional activity↓
- cell-to-cell contact↓, apoptosis↑
- vascular disruption↑: e.g. ICAM1↑, TGFB↑, PGRMC1/-2↓
- P4/PGR-driven immunomodulation
  - → proinflammatory signaling ↑(IL8-, TGFβ-, NF-κB)
- metabolic effects (↑local glucocorticoids, ↑PPARγ signaling)
- activation of fetal prostaglandin synthesis: COX2 ↑ ⇉ PGF2α↑

→ *Overlapping effects between natural and antigestagen-induced PGR-withdrawal*
→ *placentolysis*
→ *luteolysis (placental PGF2α)*

3 | EMBRYO–/FETO-MATERNAL CONTACT AND DECIDUALIZATION

According to earlier descriptions, canine embryos arrive in the uterus rather late, at approx. day 7–10 of embryonic life (Bischoff, 1845; Holst & Phemister, 1971). Even in the absence of the classical recognition of pregnancy, the embryos manifest their presence in the uterus and modulate its function in order to facilitate implantation and placentation. Interestingly, along with the lack of spontaneous P4-driven decidualization, as indicated by our studies (Graubner, Reichler, et al., 2017; Kautz et al., 2014), the biochemical changes appear to precede morphological modifications in the preimplantation uterus. Among the modulated genes are factors known as decidualization markers, for example, insulin-like growth factor 1 (*IGF1*), *PGR*, ERα/*ESR1*, prolactin receptor (*PRLR*; but not *PRL*), as well as members of the prostaglandin family, like prostaglandin transporter (*PGT*) or PGE2 synthase (*PTGES*), and PGE2 receptors, for example, *PTGER2/EP2* (Kautz et al., 2014; Kowalewski et al., 2015). As a part of this so-called predecidualization, our genomic approach indicated that further changes occur (Graubner, Gram, et al., 2017), including the modification of extracellular matrix (ECM), cell signalling, secretory activity and matrix–cell interactions characterized by cell motion and migration (Graubner, Gram, et al., 2017). The next important group of factors relates to the activation of the inflammatory response as represented in activated pathways and gene networks (Graubner, Gram, et al., 2017). Some of our recent findings regarding the involvement of the immune system in canine pregnancy (Tavares Pereira, Nowaczyk, Aslan, et al., 2021; Tavares Pereira, Nowaczyk, Payan Carreira, et al., 2021) are briefly discussed below.

Among the most interesting findings from our genomic approach, indicating the translational applicability of our observations, is the high degree of similarity in the changes associated with the presence of early preimplantation embryos (days 10–12) in the canine uterus and its human counterpart during the window of implantation (Graubner, Gram, et al., 2017). This was interpreted in the context of ongoing (pre)decidualization in preparation for implantation and trophoblast invasion (Graubner, Gram, et al., 2017).

The more intimate embryo-maternal contact starts during implantation, ultimately leading to the formation of the canine endotheliochorial placenta, with the integral development of the maternal-stroma derived decidual cells (Graubner, Reichler, et al., 2017). The canine decidual cells are morphologically clearly distinguishable and first become visible in the subepithelial compartments during the attachment of the conceptus to the uterine surface (Graubner, Reichler, et al., 2017), initializing the process of decidualization. In the mature placenta, decidual cells are located close to the maternal vessels (Kowalewski et al., 2021). They undergo mesenchymal–epithelial transition, acquiring epithelial characteristics, becoming large, increasing their secretory activity, and being surrounded by a layer of interstitial matrix, apparently containing COL4, laminins (e.g. LAMA2) and other similar substances existing in the basement membranes of epithelial compartments (Graubner et al., 2018, 2020; Kautz et al., 2015; Kowalewski et al., 2021). Concomitantly, they retain their expression of vimentin and alpha smooth muscle actin (α-SMA), both of which are mesenchymal markers (Graubner, Reichler, et al., 2017; Kautz et al., 2015). As a characteristic of endotheliochorial placentation, together with maternal endothelial cells, decidual cells evade the trophoblast invasion. Besides steroid receptors, decidual cells also express oxytocin receptor (OXTR) and RLN receptor RXFPR2 (Gram, Boos, et al., 2014; Nowak et al., 2017). Whereas the increased presence of OXTR in decidual cells during normal and antigestagen-induced luteolysis implies its involvement in the signalling cascade leading to the prepartum release of prostaglandins (Gram, Boos, et al., 2014; Figure 1), the decidualization capacity of RLN is known from human endometrial stromal cells, where it stimulates intracellular cAMP production (Telgmann et al., 1997; Telgmann & Gellersen, 1998).

4 | NATURAL VERSUS INDUCED LUTEOLYTIC CASCADE AND PRO-LUTEOLYTIC EFFECTS OF ANTIGESTAGENS

Detailed descriptions of the prepartum luteolytic cascade have already been published (Kowalewski, 2014, 2017; Kowalewski et al., 2015) and are briefly outlined above, including the involvement of the PGR expressed in decidual cells. As mentioned elsewhere, blocking the PGR functionality with antigestagens (aglepristone) evokes similar morpho-functional changes in the utero-placental compartments to those during natural luteolysis and stimulates an endocrine cascade leading to prepartum luteolysis/abortion (Baan et al., 2005, 2008; Gram et al., 2013; Kowalewski et al., 2010; Nowak et al., 2019). At the luteal level, massive luteolytic signals are observed in the CL, apparently related to the prepartum release of utero-placental PGF2α (Gram et al., 2013; Zatta et al., 2017).

The pro-luteolytic effects of antigestagens in the dog are well documented and mostly relate to the induction of the placental luteolytic cascade and antigestagen-induced placentolysis, particularly when administered during the later luteal stages. The time-dependent effects and the placental maturation towards parturition which have been recently highlighted (Nowak et al., 2019) are also discussed in greater detail below. As for the direct effects of antigestagens on luteal functionality, our transcriptomic data (Zatta et al., 2017) show global genomic changes related to the withdrawal of PGR/P4 function in the CL of dogs administered with aglepristone, compared with natural prepartum luteolysis. The P4/PGR-driven effects include inhibition of transcriptional activity, negative regulation of proliferation and protein metabolism, as well as increased immune response (albeit lower than during natural luteolysis), and apoptosis (Zatta et al., 2017). Similarly, premature luteolysis following administration of aglepristone was observed in studies by the Aslan and Schäfer-Somi group (e.g. in [Kaya et al., 2014]). Also, degenerative changes in the CL of bitches treated with aglepristone have been shown, as well as the presence of an increased number of macrophages in the CL, following aglepristone administration

(Martin et al., 2009). In another study, administration of aglepristone to non-pregnant bitches during the mid-luteal phase markedly reduced the length of P4 secretion by the CL (Polisca et al., 2010), while Gunzel-Apel et al. (2009) reported early embryonic death in bitches treated with aglepristone. Yet, the extent to which the effect observed in pregnant bitches relates to the PGR functionality directly in the CL or indirectly via activation of the placental luteolytic cascade remains to be further elucidated.

These factors must be considered when effects in nonpregnant dogs are being compared with those observed in pregnant dogs (in addition to the time point of administration during pregnancy).

5 | IN VITRO MODEL OF DECIDUALIZATION AND IMMORTALIZED CELL LINE FOR FUNCTIONAL STUDIES

We have developed an in vitro model of canine decidualization (Kautz et al., 2015) using stromal cells from naturally oestrogenized dogs (early dioestrus). Our experimental approach is based on the cAMP-mediated effects as this secondary messenger is known to exert stronger effects than P4 on endometrial stromal cells (Gellersen & Brosens, 2003; Kautz et al., 2015; Kim & Fazleabas, 2004; Tamura et al., 2012). Moreover, as mentioned elsewhere, and in contrast with humans, there is no spontaneous (i.e. P4-driven) decidualization in the dog and P4 is considered a weak inducer of decidualization (Graubner, Reichler, et al., 2017). More recently, the decidualization capacities of cAMP versus P4 in the canine model have been compared and extensively discussed (Graubner et al., 2020), confirming cAMP as a potent downstream route for the induction of decidualization in dogs, similar to other species with invasive placentation types. Addressing the need for a stable in vitro model, we have further developed our decidualization model and established an immortalized dog uterine stromal (DUS) cell line for basic research on canine decidualization (Graubner, Reichler, et al., 2017). In response to cAMP, decidualizing DUS cells express a wide spectrum of decidualization markers, for example, *IGF1*, *PRLR*, *PGR*, *PTGES*, *PTGER2* and *PTGER4* (Graubner, Reichler, et al., 2017), and enter the mesenchymal–epithelial transition, showing increased production of COL4 (Graubner et al., 2020; Figure 1). As for the P4-mediated effects, their possible direct and indirect contributions to decidualization are presented below. Interestingly, in contrast to humans and rodents, but in accordance with our previous findings, canine decidual cells do not express PRL (Kautz et al., 2015; Kowalewski, Michel, et al., 2011).

In our functional in vitro studies, we utilize type II antigestagens (aglepristone and mifepristone), which are functional competitors of P4, preventing its biological functions. The use of antigestagens in veterinary therapy, as well as in basic and clinical reproductive research, is well documented and includes our contributions (reviewed in [Kowalewski, Tavares Pereira, Papa, et al., 2020]). Type II antigestagens bind the PGR more strongly than the natural ligand (Leonhardt & Edwards, 2002), and induce its association with target DNA, but instead of recruiting transcriptional coactivators of PGR, they recruit transcriptional corepressors to promoters of target genes (Beck et al., 1996; Bocquel et al., 1993; Edwards et al., 1995; Klein-Hitpass et al., 1991; Leonhardt & Edwards, 2002). They act, therefore, as transdominant repressors inhibiting the downstream cascades (Edwards et al., 1995). From the experimental perspective, type II antigestagens do not require the presence of P4 to exert their negative effects, due to their mode of action.

6 | RECENT ADVANCEMENTS IN UNDERSTANDING ENDOCRINE REGULATION OF CANINE PREGNANCY UTILIZING IN VIVO AND IN VITRO APPROACHES

Below, some of our most recent in vivo and in vitro observations are highlighted and collectively discussed in detail. These include selected functional clues from our transcriptomic approach (Nowak et al., 2019; Tavares Pereira et al., 2022), insights into immunomodulatory effects (Tavares Pereira, Nowaczyk, Aslan, et al., 2021; Tavares Pereira, Nowaczyk, Payan Carreira, et al., 2021), and implications for the local role of glucocorticoids (Tavares Pereira et al., 2023). In particular, the in vitro studies include deeper insights into species-specific decidualization and PGR-mediated effects, as well as offering perspectives for future investigations including translational research.

6.1 | In vivo alterations in the placental feto-maternal communication

By applying deep sequencing (RNA-Seq) on placental samples from the mid-pregnant dogs and comparing them with the changes observed during normal and aglepristone-induced luteolysis, further insights have been obtained on the mechanisms of placental maturation towards parturition and on the effects of the natural and induced withdrawal of P4/PGR function (Nowak et al., 2019).

Thus, in addition to the stimulation of the luteolytic output of foetal PGF2α, the withdrawal of PGR function leads to the disturbance of placental homeostasis, mirrored in the disruption of the feto-maternal interface, including alterations in vascular function and apoptosis, as well as induction of the pro-inflammatory immune response (Nowak et al., 2019). These findings are reflected in the top overrepresented functional terms associated with prepartum luteolysis, which overlap with induced luteolysis to a great extent, including negative regulation of endothelial cell function and cell-matrix adhesion, and increased apoptosis signalling. Concomitantly, mid-gestation is characterized by increased cytokinesis, cell division and DNA replication, which are diminished by the functional withdrawal of PGR, cumulatively indicating the proliferative and anti-apoptotic properties of P4/PGR in the maintenance of placental homoeostasis. The proliferative and anti-apoptotic properties of

PGR signalling in decidual cells were recently demonstrated in our in vitro experiments (Kazemian et al., 2022). The transcriptional and DNA replication-associated positive effects of PGR underline its function as a nuclear receptor and transcriptional factor, with the antigestagen-mediated effects indicating its downstream molecular targets. An even stronger emphasis on the importance of the functional withdrawal of P4/PGR for the initialization of parturition is provided by P4 and PGR being listed as the top upstream regulators during normal and induced luteolysis (Nowak et al., 2019; Figure 1).

Other predicted top upstream regulators are dexamethasone, PPARγ and TGFβ (Nowak et al., 2019). The presence of dexamethasone among the important regulators of the luteolytic process drew our attention as it points towards an involvement of glucocorticoids in the luteolytic cascade in the dog. This relatively poorly understood area of canine reproductive physiology has been addressed in more detail in our recent studies (discussed below and in [Tavares Pereira et al., 2023]). PPARγ is an interesting regulatory candidate expressed in the foetal trophoblast, and its importance in the maintenance of canine pregnancy was discussed previously (Kowalewski, Meyer, et al., 2011). It serves as an alternative intracellular receptor for arachidonic acid metabolites (including prostaglandins; Berger & Moller, 2002) and was implicated in regulating trophoblast invasion during invasive placentation in other species (Asami-Miyagishi et al., 2004; Barak et al., 1999). Based on its response to the withdrawal of PGR function, PPARγ seems to be regulated by the P4–PGR interaction (Kowalewski, Meyer, et al., 2011). Finally, TGFβ and its associated factors, for example, *TGFB3*, *TGFBR1* and *TGFBR2*, were highly expressed during luteolysis (Nowak et al., 2019). TGFβ is a highly versatile, multifunctional factor that was earlier implicated in regulating luteolysis in cattle, including the release of foetal membranes and the disruption of luteal vascular integrity (Maroni & Davis, 2011; Massague, 2012). The signalling pathways corresponding to the detected upstream regulators were also predicted to be jointly activated in the placenta during normal and induced luteolytic cascade.

Among the genes affected during prepartum luteolysis, 127 were predicted to be regulated by P4 (Nowak et al., 2019). This list involved factors responsible for metabolic, vascular and immune function, for example, *ICAM1* regulating vascular stability, integrity and permeability of vessels, or *PTGS2* (*COX2*), *PRLR*, *VEGF* and PGE2 receptor *PTGER2*, the functional involvement of which in maintaining placental homeostasis in the dog has previously been addressed (Gram et al., 2015; Gram, Fox, et al., 2014; Kowalewski et al., 2010; Kowalewski, Michel, et al., 2011). Several of the P4-dependent genes overlapped between normal and induced parturition (Nowak et al., 2019), for example, some metalloproteinases and *HSD11B2* (Nowak et al., 2019; Figure 1). The presence of *HSD11B2* among the P4-regulated genes in natural and antigestagen-mediated withdrawal of P4 was of special interest as its gene product is an enzyme responsible for the conversion of cortisol to cortisone, and thereby for inactivating cortisol. Its transcriptional levels were suppressed during luteolysis, indicating increased local availability of placental cortisol upon withdrawal of P4/PGR signalling (Nowak et al., 2019).

In subsequent studies, the canine uterine/utero-placental spatio-temporal expression of HSD11B2 throughout pregnancy was assessed using custom-made species-specific antibodies, confirming the decreased availability of HSD11B2 at prepartum luteolysis and its localization in foetal trophoblast (Tavares Pereira et al., 2023). Concomitantly, placental microsomes obtained from placental samples during prepartum luteolysis showed reduced capacity to deactivate cortisol into cortisone. This finding fits well with the diminished HSD11B2 levels and suggests increased placental availability of cortisol at term, perhaps directed towards increased expression of GR/NR3C1 in the trophoblast (Gram et al., 2016). With this, the canine placenta appears to actively regulate its own local cortisol levels during prepartum luteolysis. The possible role of cortisol, unrelated to its variable peripheral levels (itself representing an important clinical feature), could be in regulating the availability of its own GR/NR3C1 receptor. The role of the latter was proposed in regulating the local availability of P4 by functional competition with PGR for its natural ligand (Gram et al., 2016; Tavares Pereira et al., 2023), similar to that in humans (Karalis et al., 1996; Ojasoo et al., 1988). Other proposed roles of cortisol could be in regulating the maturation of foetal organs or the local availability of prostaglandins (Tavares Pereira et al., 2023).

6.1.1 | Normal versus induced luteolysis

Interestingly, despite the similarities observed in placental transcriptional signatures and associated signalling pathways, we showed that there were important differences in the effects evoked by the natural and induced withdrawal of PGR, as reflected in a high number of differentially expressed genes between these two groups (Nowak et al., 2019). Despite being strongly represented under both conditions compared with mid-gestation, several factors prevailed during the natural withdrawal of PGR, representing functional terms related to the negative regulation of angiogenesis, immune system-related processes and cytokine production, and including, for example, pathways related to IL8-, TGFβ-, NF-κB-, or PKA-signalling (Nowak et al., 2019). Since our experiments with antigestagens were performed using mid-term dogs, it is possible that placental maturation (Nowak et al., 2019), and hence preparation for parturition, could explain the observed differences between the normal and induced luteolytic cascades. Similar conclusions were drawn from our recent investigations of the involvement of the immune system during normal and induced parturition, showing an upregulated, though to some extent differently modulated, placental immune response under both conditions (Tavares Pereira, Nowaczyk, Aslan, et al., 2021).

It also appears plausible that, as a part of placental maturation, the functional priming of the placenta towards term by continuously decreasing circulating luteal levels of P4, and thereby decreasing P4-PGR signalling, could play an important role. Following this line, our recent, as yet unpublished, study indicated the decreasing number of decidual cells as a possible contributor to the diminishing PGR

signalling prior to term (Figure 1). As discussed elsewhere (Nowak et al., 2019), our findings agree with clinical observations, indicating important differences between normal and antigestagen-induced luteolysis (Baan et al., 2008; Fieni et al., 2001; Galac et al., 2000; Hoffmann et al., 1999). PTGS2/COX2 was among the factors differently expressed under normal and antigestagen-induced luteolysis, but upregulated compared with mid-gestation controls, which could relate to clinical findings of differing circulating peripheral PGF2α under both conditions, which are lower following treatment with antigestagens (Kowalewski et al., 2010; Nohr et al., 1993). These differences should be considered when the clinical application of antigestagens is considered, as their effects may be time dependent.

Significant new information on the immunomodulatory effects at the embryo-maternal interface and the regulatory properties of P4/PGR signalling upon the immune response was reported recently (Tavares Pereira, Nowaczyk, Aslan, et al., 2021; Tavares Pereira, Nowaczyk, Payan Carreira, et al., 2021). Accordingly, moderated pro-inflammatory signals seem to prevail during the establishment of pregnancy, while implantation and early trophoblast invasion (thus, early decidualization) appear to be associated with an immunomodulatory and rather anti-inflammatory environment (Tavares Pereira, Nowaczyk, Payan Carreira, et al., 2021). Supported by the transcriptomic data, termination of pregnancy is associated with increased activity of M2a and M2c macrophages as well as Treg and Th lymphocytes, paralleled by their increased placental presence at term (Tavares Pereira, Nowaczyk, Aslan, et al., 2021). Despite several similarities, the transcriptional availability of some immune system-related factors differed between the natural and induced PGR withdrawal, indicating an even higher level of M1 macrophages following antigestagen treatment and a higher incidence of NK cells than in samples collected at term (Tavares Pereira, Nowaczyk, Aslan, et al., 2021). Therefore, besides placental maturation, the transdominant repressor activity of type II antigestagens could also play a role leading to clinical implications in the management of bitches in which preterm parturition/abortion is induced with antigestagens (Tavares Pereira, Nowaczyk, Aslan, et al., 2021).

6.2 | In vitro functional studies; extracellular matrix (ECM) and cell-to-cell communication, progesterone-, PGE2- and antigestagen-mediated effects, followed by in-depth transcriptome analysis using NGS

In vitro decidualization of the DUS cell line via cAMP resulted in the increased expression of COL4, ECM1 and of the gap junction molecule CX43, in parallel with vimentin and α-SMA expression, thereby confirming the mesenchymal–epithelial transition of canine uterine stromal cells typical of other decidualization models associated with invasive placentation (e.g. humans; Graubner et al., 2020; Kazemian et al., 2022; Figure 1).

Moreover, we proved that PGE2-induced decidualization is mediated through its cAMP-signalling receptors (PTGER2/4), and PGE2 stimulates the expression of *PGR* during decidualization (as well as *PRLR*) in a PTGER2/4-dependent manner (Graubner et al., 2020). Reciprocally, PGR regulates the availability of PGE2 synthase (PTGES) in decidualizing DUS cells (Kazemian et al., 2022), while P4 appears to be involved in regulating the availability of PGE2 receptors (Graubner et al., 2020). Furthermore, in a self-regulatory loop, PGE2 increases the expression of its own synthase (*PTGES*) and *PTGER4* (Graubner et al., 2020). On the other hand, however, despite inducing the expression of decidualization markers, PGE2 does not modulate the expression of ECM related factors, for example, COL4 and ECM1 (Graubner et al., 2020), and thus, does not seem to be involved in the mesenchymal-epithelial transition. As shown recently using the DUS cell model, the mesenchymal–epithelial transition seems to be under PGR control, as the application of antigestagens in a transcriptomic approach reversed the expression of DEGs associated with regulation of the transition (Tavares Pereira et al., 2022).

As an important conclusion from our in vitro findings, although P4 is considered a weaker spontaneous inducer of decidualization in the dog in vivo, in our DUS cell model, it was capable of upregulating the expression of some decidualization markers and its own PGR receptor, thereby revealing a basic decidualization capacity (Graubner et al., 2020). With this, the in vitro studies imply both direct (by regulating the availability of PGR), and indirect (PGE2-mediated), involvement of P4-dependent pathways in canine decidualization (Graubner et al., 2020). In support of this, in our investigations into antigestagen-mediated effects in decidualized DUS cells, antigestagens (aglepristone and mifepristone) suppressed expression of decidualization markers, including PGR, and the proliferative and anti-apoptotic properties of PGR in decidual cells were substantiated (Kazemian et al., 2022).

Another important observation from the in vitro study were the effects exerted by the diminished PGR signalling upon the expression of the gap-junctional *CX43*, suggesting that its decreased availability during normal and induced luteolysis could additionally affect the cell-to-cell interaction between decidual cells and their neighbouring cellular compartments, including the trophoblast, additionally contributing to disturbances in placental homeostasis and integrity (Kazemian et al., 2022), as had already been suggested by our transcriptomic study (Nowak et al., 2019).

Adding new cellular and molecular targets to P4-mediated effects, recent data from our laboratory on the presence and possible functions of membrane-bound P4 receptors in canine placenta and DUS cells indicated distinct distribution patterns of membrane receptors within the canine placenta: PGRMC2 was predominantly localized in decidual cells, PGRMC1 was mostly represented in maternal endothelial cells, and syncytiotrophoblast showed abundant levels of mPRα and mPRβ (encoded by *PAQR7* and *−8*, respectively; Kazemian et al., 2023; Figure 1). Thus far, the P4-mediated effects in the canine placenta have been attributed to the direct canonical/genomic effects of P4 through its decidual cell-associated PGR. These new findings indicate, however, the direct involvement of non-canonical/non-genomic pathways of P4 signalling in the canine placenta, acting on different cellular compartments, both maternal

and foetal (Figure 1). Additionally, as shown using the DUS cell line, the indirect effects of PGR appear to apply, as antigestagens strongly reduced PGRMC2 (and PGRMC1) expression. With this, the PGR appears to regulate not only its own availability (Kazemian et al., 2022), but also, indirectly, the P4-mediated activity of decidual cells by affecting the expression of membrane-bound receptors. These recent findings shed a new light on P4 functionality within the canine placenta, which appears to include possible effects of vascular tonus and integrity, and possibly trophoblast invasiveness mediated through the non-canonical signalling pathways. Together with the previous observations on prepartum luteolysis being associated with the disruption of placental cell-to-cell communication, vascular integrity and increased immune response (Nowak et al., 2019), the possible effects of membrane-bound receptors, for example, PGRMC1, in endothelial cells and their dependence upon PGR are of particular interest. Although plausible, the biological properties of membrane-bound receptors in various cellular compartments of the canine placenta, including decidual cells, remain to be verified.

6.2.1 | Genome-wide transcriptional effects in DUS cells and implications for canine decidualization as a model for better understanding placental disturbances in humans

The transcriptome signature of DUS cells was assessed and, together with the effects mediated by type II antigestagens, provided new clues regarding the decidualization process in the dog (Tavares Pereira et al., 2022). Based on the overrepresented functional terms, the mechanism of canine-specific decidualization consists of cellular proliferation and adhesion, epithelization and mesenchymal–epithelial transition, extracellular matrix organization, and vaso- and immunomodulation. Besides PKA, which was expected due to the cAMP-mediated decidualization approach, selected MAPK pathways and cyclins were also included in the overrepresented canonical pathways. Fitting the previous findings, the predicted upstream regulators included P4, PGE2, PGR, PRL/PRLR and VEGFA. Concomitantly, the functional terms associated with apoptosis were underrepresented, supporting the negative regulation of apoptotic events during decidualization (Tavares Pereira et al., 2022). Conversely, antigestagens greatly suppressed the effects of cAMP, including deactivation of PKA signalling and reinforcing the negative effects of antigestagens upon proliferation, concomitantly enhancing apoptosis and suppressing cell migration, motility and angiogenic properties (Figure 1).

In relation to the morpho-functional transition of the cells, there was an increased presence of some BMP family members in association with Wnt signalling, linked previously to decidualization and implantation (Lee et al., 2007; Monsivais et al., 2017). The Wnt-related factors were represented by cell adhesion molecules, including selectin P (SELP), which was one of the most upregulated differentially expressed genes in decidualized cells. The function of SELP spans from increasing the immune cell infiltrate (Zenclussen et al., 2001) to being associated with other selectins in support of uterine receptivity and the implantation process (Feng et al., 2017; Genbacev et al., 2003). Importantly, SELP has been implicated in preeclampsia, a life-threatening condition in humans associated with placenta-derived hypertension (Lok et al., 2007; Mistry et al., 2020). Although the multifactorial pathophysiology of preeclampsia is not fully understood, and both maternal (aberrant decidualization) and foetal (aberrant trophoblast invasion) origins are discussed, it is associated with deficient vascularization of the placenta and shallow invasion of the trophoblast that leads to maternal high blood pressure and renal dysfunction, among other conditions (Phipps et al., 2019). In our data set, the vasoactive effects of decidualization were also associated with the modulation of angiogenic factors; for example, we observed opposing effects on the expression of the endothelin receptors *EDNRB* and *EDNRA*, whereas *EDNRB* was decreased, the availability of *EDNRA* was increased (Tavares Pereira et al., 2022). Endothelins are involved in the regulation of vasodilation/constriction, with the receptor B (encoded by *EDNRB*) being known as a potent vasodilator, and receptor A (encoded by *ENDRA*) acting as a potent vasoconstrictor (Schneider et al., 2007). We also observed an increased expression of the activator of endothelins, endothelin-converting enzyme 2 (*ECE2*), in decidualized cells. Interestingly, endothelins are also associated with the onset of preeclampsia in humans (Granger et al., 2018). Another factor frequently associated with preeclampsia is the soluble form (alternative splice variant) of VEGFR1/FLT1, sFLT1, acting as an antiangiogenic factor (Ives et al., 2020; Kendall & Thomas, 1993; Maynard et al., 2003; Palmer et al., 2017; Phipps et al., 2019; Sircar et al., 2015). Its production is decreased in primary human endometrial stromal cells after in vitro decidualization (Cottrell et al., 2017). Although the sFLT1 isoform was not among the DEG in our data set, the expression of *VEGFR1* (*FLT1*) was downregulated in response to decidualization in DUS cells. Other vasomodulatory factors differentially expressed after DUS cell decidualization included increased transcript levels of *THBS2* and downregulated *ANGPT4* (Tavares Pereira et al., 2022). Both angiopoietins and thrombospondins, in particular THBS1, have been implicated in aberrant trophoblast invasion in humans (Apostolakis et al., 2009; McElrath et al., 2020; Ulu et al., 2019). Contrasting with the increased expression of *FLT1*, both *SELP* and *THBS2* were among the genes strongly decreased by antigestagen-mediated withdrawal of PGR in DUS cells. In summary, the observed expression patterns of specific vascular factors provide additional evidence supporting the connection between decidualized cells and vascular remodelling, as well as placental blood flow in dogs, adding to the possible interaction between the PGR and membrane-bound P4 receptors in regulating decidual cell function. Besides the apparent effects upon the vascular factors, type II antigestagens greatly reversed the cAMP-induced decidualization effects. Similar top upstream regulators were found in antigestagen-treated cells, corresponding to those identified in the placental isolates discussed above (Nowak et al., 2019). Concomitantly, decreased prostaglandin signalling was found in DUS cells. Among the factors involved in cellular growth, annexin A2 (ANXA2), in particular, attracted our

attention. Deficiency of decidua-derived ANXA2 impairs decidualization and results in shallow decidual trophoblast invasion in women, contributing to placental abnormalities, which have been linked to the development of severe preeclampsia (Garrido-Gomez et al., 2020, 2022). Interestingly, in our data set obtained with decidualized DUS from the dog, that is, a species with physiologically shallow trophoblast invasion, there was a lowered expression of *ANXA2*, which became elevated in response to antigestagens. Thus, decreased ANXA2 appears to be characteristic of shallow invasion, both under physiological conditions in the dog and during pathologically shallow invasion in humans. Consequently, adding to the information implicating SELP and vasoactive factors in preeclampsia, it appears plausible that the canine model could be utilized to create a better understanding of the aberrant shallow trophoblast invasion in humans.

By comparing the effects induced by both type II antigestagens and transcriptional alterations in the canine placenta at term (Nowak et al., 2019), a significant number of DEGs, potentially indicating the signalling pathways associated in vivo with decidual cells and implicated in the termination of canine pregnancy, were identified (Tavares Pereira et al., 2022). When both type II antigestagens were considered, a large number of these DEGs included 191 target genes. However, taking into account the diverging effects evoked by either of the antigestagens, 243 and 262 DEGs were identified for aglepristone and mifepristone, respectively, overlapping with genes induced by natural luteolysis. The overrepresented DEGs involved biological processes related to cell migration and proliferation, negative regulation of cellular response to growth factor stimulus, regulation of the mesenchymal–epithelial transition, angiogenesis and apoptosis, as well as negative regulation of the transmembrane receptor protein serine/threonine kinase signalling pathway. Some of the genes represented have already been mentioned (*SELP*, *THSB2*, *ENDRB* and *FLT1*), but the list also included *VEGFA*, the proapoptotic *BCL2*, the integrin receptor-related subunit *ITGA*, as well as *AKAP12* and *CDKN1A*. The latter two factors, that is, AKAP12 and CDKN1A, are linked to kinase activity, with AKAP12, that is, A-kinase anchoring protein 12, being a scaffold protein in signal transduction. As an anchoring protein, it tethers cAMP-related kinases, including PKA and PKC, to subcellular compartments for better coordination of their functions (Beal et al., 2021; Michel & Scott, 2002; Nelson et al., 1999). Its expression was induced by decidualization in DUS cells. CDKN1A, cyclin-dependent kinase inhibitor 1A, regulates the activity of various cell cycle-related kinases (Bendjennat et al., 2003; Kreis et al., 2019).

Linking PGR function to kinase activity was indeed an interesting finding which attracted our attention. We found that several kinases and their related proteins were negatively targeted at the transcriptional level by antigestagens in decidualized DUS cells, including the suppressed expression of *AKAP12*, *CAMK1D* (calcium/calmodulin dependent protein kinase), some of the cyclin-dependent kinases, for example, *CDK16*, *IRAK2* (interleukin 1 receptor-associated kinase 2), *ITPKB* and *-C* (inositol-trisphosphate 3-kinase B, and -C), as well as the functional component of the PKA, *PRKAR1A* (protein kinase cAMP-dependent type I regulatory subunit alpha) (Tavares Pereira et al., 2022). The presence of *PRKAR1A* among the suppressed genes could contribute to the decreased PKA activity during the natural prepartum luteolysis and in antigestagen-treated DUS cells, implying that the reduced signalling of these and other kinases could be related to their transcriptional availability. It appears, thus, plausible that the withdrawal of PGR function could have a direct regulatory effect on cAMP/PKA signalling in decidualized cells by targeting gene transcription. As already noted, the negative effects observed with antigestagen treatment could be related to their transdominant repressor activity. Further implication of PGR-mediated regulation of PKA activity arises from the interaction between PGR and PGE2 pathways and EP2/EP4 receptors acting via the cAMP/PKA pathway (Woodward et al., 2011). Thus, depriving the PGE2 receptors of their specific kinases could hamper their downstream effects.

Following on from the involvement of kinases in PGR-mediated effects, we are currently applying a novel kinomics technology in an ongoing study in which we have been able to predict the activity of serine/threonine kinases involved in regulating decidualization in the dog. The technology used is based on the PamChip assay (PamGene International BV) and is a merger between genomics and proteomics. Our group was the first to apply this technology to canine research. The data generated indicate that the downstream signalling cascades involve diverse kinetic pathways that are functionally modified both during decidualization and in response to antigestagens. Over 80 serine/threonine kinases were predicted to be activated in decidualized cells, besides PKA, including other ACG kinases (i.e. several PKG and PKC kinases), CK1 (casein kinases, linked to Wnt signalling), CAMK (Ca2+/calmodulin-dependent protein kinases), CMGC including cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs and ERKs), JNK kinases, glycogen synthase kinases (GSKs) and CDC-like kinase (CLKs). The activity of virtually all the activated kinases was affected by treatment with type II antigestagens (unpublished). Studies dissecting the activity of some of the kinases and their involvement in regulating decidualization are ongoing.

7 | SUMMARY AND OUTLOOK

We have extensively discussed various observations, and our most significant discoveries pertain to the specific molecular and endocrine mechanisms that facilitate communication between the developing canine embryo and the mother (Figure 1). The main focus has been on placental decidual cells, and the effects mediated by PGR. Consequently, several possible molecular targets for future research have been proposed within the canine placenta. With regard to P4, they involve both canonical and, newly, non-canonical responses. Our research has also laid a foundation for further investigation into the immune system's role in the canine parturition cascade, highlighting the immunomodulatory effects of P4 in the utero-placental compartments, apparently mediated through PGR-expressing decidual cells. By establishing the DUS cell model, we have created a

unique tool for exploring and better understanding canine decidualization and the effects on maternal decidual cells following the withdrawal of P4/PGR function, paving the way for targeted downstream studies.

These findings offer a basis for future research into feto-maternal communication in dogs, supporting the development of improved clinical protocols for the control of canine reproduction. Lastly, we have discussed canine decidualization as a potential model for translational studies on aberrant trophoblast invasion observed in humans.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

Apostolakis, S., Shantsila, E., & Lip, G. Y. (2009). Angiopoietins and pre-eclampsia: New perspectives in the quest for markers. *American Journal of Hypertension*, *22*(8), 820. https://doi.org/10.1038/ajh.2009.108

Asami-Miyagishi, R., Iseki, S., Usui, M., Uchida, K., Kubo, H., & Morita, I. (2004). Expression and function of PPARgamma in rat placental development. *Biochemical and Biophysical Research Communications*, *315*(2), 497–501. https://doi.org/10.1016/j.bbrc.2004.01.074

Baan, M., Taverne, M. A., de Gier, J., Kooistra, H. S., Kindahl, H., Dieleman, S. J., & Okkens, A. C. (2008). Hormonal changes in spontaneous and aglepristone-induced parturition in dogs. *Theriogenology*, *69*(4), 399–407. https://doi.org/10.1016/j.theriogenology.2007.10.008

Baan, M., Taverne, M. A., Kooistra, H. S., de Gier, J., Dieleman, S. J., & Okkens, A. C. (2005). Induction of parturition in the bitch with the progesterone-receptor blocker aglepristone. *Theriogenology*, *63*(7), 1958–1972. https://doi.org/10.1016/j.theriogenology.2004.09.008

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A., & Evans, R. M. (1999). PPAR gamma is required for placental, cardiac, and adipose tissue development. *Molecular Cell*, *4*(4), 585–595.

Beal, R., Alonso-Carriazo Fernandez, A., Grammatopoulos, D. K., Matter, K., & Balda, M. S. (2021). ARHGEF18/p114RhoGEF coordinates PKA/CREB signaling and Actomyosin remodeling to promote trophoblast cell-cell fusion during placenta morphogenesis. *Frontiers in Cell and Development Biology*, *9*, 658006. https://doi.org/10.3389/fcell.2021.658006

Beck, C. A., Zhang, Y., Weigel, N. L., & Edwards, D. P. (1996). Two types of anti-progestins have distinct effects on site-specific phosphorylation of human progesterone receptor. *The Journal of Biological Chemistry*, *271*(2), 1209–1217.

Bendjennat, M., Boulaire, J., Jascur, T., Brickner, H., Barbier, V., Sarasin, A., Fotedar, A., & Fotedar, R. (2003). UV irradiation triggers ubiquitin-dependent degradation of p21(WAF1) to promote DNA repair. *Cell*, *114*(5), 599–610. https://doi.org/10.1016/j.cell.2003.08.001

Berger, J., & Moller, D. E. (2002). The mechanisms of action of PPARs. *Annual Review of Medicine*, *53*, 409–435. https://doi.org/10.1146/annurev.med.53.082901.104018

Bischoff, T. L. W. (1845). *Entwicklungsgeschichte des Hunde-Eies. (Eng.: The development of the canine oocyte.)*. Druck und Verlag von Friedrich Veweg und Sohn. http://digi.ub.uni-heidelberg.de/diglit/bischoff1845/0005?sid=d04cee3b21867da2b18620756659777e

Bocquel, M. T., Ji, J., Ylikomi, T., Benhamou, B., Vergezac, A., Chambon, P., & Gronemeyer, H. (1993). Type II antagonists impair the DNA binding of steroid hormone receptors without affecting dimerization. *The Journal of Steroid Biochemistry and Molecular Biology*, *45*(4), 205–215. https://doi.org/10.1016/0960-0760(93)90334-s

Concannon, P. W., McCann, J. P., & Temple, M. (1989). Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *Journal of Reproduction and Fertility. Supplement*, *39*, 3–25.

Cottrell, H. N., Wu, J., Rimawi, B. H., Duran, J. M., Spencer, J. B., Sidell, N., & Rajakumar, A. (2017). Human endometrial stromal cell plasticity: Reversible sFlt1 expression negatively coincides with decidualization. *Hypertension in Pregnancy*, *36*(2), 204–211. https://doi.org/10.1080/10641955.2017.1299172

Edwards, D. P., Altmann, M., DeMarzo, A., Zhang, Y., Weigel, N. L., & Beck, C. A. (1995). Progesterone receptor and the mechanism of action of progesterone antagonists. *The Journal of Steroid Biochemistry and Molecular Biology*, *53*(1–6), 449–458.

Feng, Y., Ma, X., Deng, L., Yao, B., Xiong, Y., Wu, Y., Wang, L., Ma, Q., & Ma, F. (2017). Role of selectins and their ligands in human implantation stage. *Glycobiology*, *27*(5), 385–391. https://doi.org/10.1093/glycob/cwx009

Fieni, F., Marnet, P. G., Martal, J., Siliart, B., Touzeau, N., Bruyas, J. F., & Tainturier, D. (2001). Comparison of two protocols with a progesterone antagonist aglepristone (RU534) to induce parturition in bitches. *Journal of Reproduction and Fertility. Supplement*, *57*, 237–242.

Galac, S., Kooistra, H. S., Butinar, J., Bevers, M. M., Dieleman, S. J., Voorhout, G., & Okkens, A. C. (2000). Termination of mid-gestation pregnancy in bitches with aglepristone, a progesterone receptor antagonist. *Theriogenology*, *53*(4), 941–950. https://doi.org/10.1016/S0093-691X(00)00241-7

Garrido-Gomez, T., Castillo-Marco, N., Cordero, T., & Simon, C. (2022). Decidualization resistance in the origin of preeclampsia. *American Journal of Obstetrics and Gynecology*, *226*(2S), S886–S894. https://doi.org/10.1016/j.ajog.2020.09.039

Garrido-Gomez, T., Quinonero, A., Dominguez, F., Rubert, L., Perales, A., Hajjar, K. A., & Simon, C. (2020). Preeclampsia: A defect in decidualization is associated with deficiency of Annexin A2. *American Journal of Obstetrics and Gynecology*, *222*(4), 376 e371. https://doi.org/10.1016/j.ajog.2019.11.1250

Gellersen, B., & Brosens, J. (2003). Cyclic AMP and progesterone receptor cross-talk in human endometrium: A decidualizing affair. *The Journal of Endocrinology*, *178*(3), 357–372.

Genbacev, O. D., Prakobphol, A., Foulk, R. A., Krtolica, A. R., Ilic, D., Singer, M. S., Yang, Z. Q., Kiessling, L. L., Rosen, S. D., & Fisher, S. J. (2003). Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. *Science*, *299*(5605), 405–408. https://doi.org/10.1126/science.1079546

Gram, A., Boos, A., & Kowalewski, M. P. (2014). Uterine and placental expression of canine oxytocin receptor during pregnancy and normal and induced parturition. *Reproduction in Domestic Animals*, *49*(Suppl 2), 41–49. https://doi.org/10.1111/rda.12295

Gram, A., Buchler, U., Boos, A., Hoffmann, B., & Kowalewski, M. P. (2013). Biosynthesis and degradation of canine placental prostaglandins: Prepartum changes in expression and function of prostaglandin F2alpha-synthase (PGFS, AKR1C3) and 15-hydroxyprostaglandin dehydrogenase (HPGD). *Biology of Reproduction*, *89*(1), 2. https://doi.org/10.1095/biolreprod.113.109918

Gram, A., Fox, B., Buchler, U., Boos, A., Hoffmann, B., & Kowalewski, M. P. (2014). Canine placental prostaglandin E2 synthase: Expression, localization, and biological functions in providing substrates for Prepartum PGF2alpha synthesis. *Biology of Reproduction*, *91*(6), 154. https://doi.org/10.1095/biolreprod.114.122929

Gram, A., Hoffmann, B., Boos, A., & Kowalewski, M. P. (2015). Expression and localization of vascular endothelial growth factor a (VEGFA) and its two receptors (VEGFR1/FLT1 and VEGFR2/FLK1/KDR) in the canine corpus luteum and utero-placental compartments during pregnancy and at normal and induced parturition. *General and Comparative Endocrinology*, *223*, 54–65. https://doi.org/10.1016/j.ygcen.2015.09.020

Gram, A., Trachsel, A., Boos, A., & Kowalewski, M. P. (2016). Elevated utero/placental GR/NR3C1 is not required for the induction of parturition in the dog. *Reproduction*, *152*(4), 303–311. https://doi.org/10.1530/REP-16-0213

Granger, J. P., Spradley, F. T., & Bakrania, B. A. (2018). The endothelin system: A critical player in the pathophysiology of preeclampsia. *Current Hypertension Reports*, *20*(4), 32. https://doi.org/10.1007/s11906-018-0828-4

Graubner, F. R., Boos, A., Aslan, S., Kucukaslan, I., & Kowalewski, M. P. (2018). Uterine and placental distribution of selected extracellular matrix (ECM) components in the dog. *Reproduction*, *155*(5), 403–421. https://doi.org/10.1530/REP-17-0761

Graubner, F. R., Gram, A., Kautz, E., Bauersachs, S., Aslan, S., Agaoglu, A. R., Boos, A., & Kowalewski, M. P. (2017). Uterine responses to early pre-attachment embryos in the domestic dog and comparisons with other domestic animal species. *Biology of Reproduction*, *97*(2), 197–216. https://doi.org/10.1093/biolre/iox063

Graubner, F. R., Pereira, M. T., Boos, A., & Kowalewski, M. P. (2020). Canine decidualization in vitro: Extracellular matrix modification, progesterone mediated effects and selective blocking of prostaglandin E2 receptors. *The Journal of Reproduction and Development*., *66*, 319–329. https://doi.org/10.1262/jrd.2019-157

Graubner, F. R., Reichler, I. M., Rahman, N. A., Payan-Carreira, R., Boos, A., & Kowalewski, M. P. (2017). Decidualization of the canine uterus: From early until late gestational in vivo morphological observations, and functional characterization of immortalized canine uterine stromal cell lines. *Reproduction in Domestic animals*, *52*(Suppl 2), 137–147. https://doi.org/10.1111/rda.12849

Gunzel-Apel, A. R., Hoftmann, T., Nottorf, S., Politt, E., Meyer-Lindenberg, A., Hoppen, H. O., Einspanier, A., Knijn, H. M., & Mischke, R. (2009). Influence of progesterone withdrawal on pregnancy-related parameters during post-implantation early pregnancy loss. *Reproduction in Domestic Animals*, *44*(Suppl 2), 174–181. https://doi.org/10.1111/j.1439-0531.2009.01381.x

Hoffmann, B., Busges, F., Engel, E., Kowalewski, M. P., & Papa, P. (2004). Regulation of corpus luteum-function in the bitch. *Reproduction in Domestic Animals*, *39*(4), 232–240. https://doi.org/10.1111/j.1439-0531.2004.00508.x

Hoffmann, B., Hoveler, R., Nohr, B., & Hasan, S. H. (1994). Investigations on hormonal changes around parturition in the dog and the occurrence of pregnancy-specific non conjugated oestrogens. *Experimental and Clinical Endocrinology*, *102*(3), 185–189. https://doi.org/10.1055/s-0029-1211280

Hoffmann, B., Riesenbeck, A., Schams, D., & Steinetz, B. (1999). Aspects on hormonal control of normal and induced parturition in the dog. *Reproduction in Domestic Animals*, *34*, 219–226.

Holst, P. A., & Phemister, R. D. (1971). The prenatal development of the dog: Preimplantation events. *Biology of Reproduction*, *5*(2), 194–206.

Ives, C. W., Sinkey, R., Rajapreyar, I., Tita, A. T. N., & Oparil, S. (2020). Preeclampsia-pathophysiology and clinical presentations: JACC state-of-the-art review. *Journal of the American College of Cardiology*, *76*(14), 1690–1702. https://doi.org/10.1016/j.jacc.2020.08.014

Karalis, K., Goodwin, G., & Majzoub, J. A. (1996). Cortisol blockade of progesterone: A possible molecular mechanism involved in the initiation of human labor. *Nature Medicine*, *2*(5), 556–560.

Kautz, E., de Carvalho Papa, P., Reichler, I. M., Gram, A., Boos, A., & Kowalewski, M. P. (2015). In vitro decidualisation of canine uterine stromal cells. *Reproductive Biology and Endocrinology*, *13*, 85. https://doi.org/10.1186/s12958-015-0066-4

Kautz, E., Gram, A., Aslan, S., Ay, S. S., Selcuk, M., Kanca, H., Koldaş, E., Akal, E., Karakaş, K., Findik, M., Boos, A., & Kowalewski, M. P. (2014). Expression of genes involved in the embryo-maternal interaction in the early-pregnant canine uterus. *Reproduction*, *147*(5), 703–717. https://doi.org/10.1530/REP-13-0648

Kaya, D., Kucukaslan, I., Agaoglu, A. R., Ay, S. S., Schafer-Somi, S., Emre, B., Bal, Y., Einspanier, A., Gürcan, I. S., Gültiken, N., & Aslan, S. (2014). The effects of aglepristone alone and in combination with cloprostenol on hormonal values during termination of mid-term pregnancy in bitches. *Animal Reproduction Science*, *146*(3–4), 210–217. https://doi.org/10.1016/j.anireprosci.2014.03.002

Kazemian, A., Tavares Pereira, M., Aslan, A., Payan-Carreira, R., Reichler, I. M., Agaoglu, R. A., & Kowalewski, M. P. (2023). Membrane-bound progesterone receptors in the canine uterus and placenta; possible targets in the maintenance of pregnancy. *Theriogenology*, *210*, 68–83. https://doi.org/10.1016/j.theriogenology.2023.07.005

Kazemian, A., Tavares Pereira, M., Hoffmann, B., & Kowalewski, M. P. (2022). Antigestagens mediate the expression of decidualization markers, extracellular matrix factors and connexin 43 in Decidualized dog uterine stromal (DUS) cells. *Animals (Basel)*, *12*(7), 798. https://doi.org/10.3390/ani12070798

Kendall, R. L., & Thomas, K. A. (1993). Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(22), 10705–10709. https://doi.org/10.1073/pnas.90.22.10705

Kim, J. J., & Fazleabas, A. T. (2004). Uterine receptivity and implantation: The regulation and action of insulin-like growth factor binding protein-1 (IGFBP-1), HOXA10 and forkhead transcription factor-1 (FOXO-1) in the baboon endometrium. *Reproductive Biology and Endocrinology*, *2*, 34. https://doi.org/10.1186/1477-7827-2-34

Klein-Hitpass, L., Cato, A. C., Henderson, D., & Ryffel, G. U. (1991). Two types of antiprogestins identified by their differential action in transcriptionally active extracts from T47D cells. *Nucleic Acids Research*, *19*(6), 1227–1234. https://doi.org/10.1093/nar/19.6.1227

Kowalewski, M. P. (2014). Luteal regression vs. prepartum luteolysis: Regulatory mechanisms governing canine corpus luteum function. *Reproductive Biology*, *14*(2), 89–102. https://doi.org/10.1016/j.repbio.2013.11.004

Kowalewski, M. P. (2017). Regulation of Corpus luteum function in the domestic dog (*Canis familiaris*) and comparative aspects of luteal function in the domestic cat (*Felis catus*). In R. Meidan (Ed.), *The life cycle of the Corpus luteum* (pp. 133–157). Springer International Publishing.

Kowalewski, M. P. (2018). Selected comparative aspects of canine female reproductive physiology. In K. Skinner Michael (Ed.), *Encyclopedia of reproduction* (2nd ed., pp. 682–691). Academic Press, Elsevier.

Kowalewski, M. P., Beceriklisoy, H. B., Pfarrer, C., Aslan, S., Kindahl, H., Kucukaslan, I., & Hoffmann, B. (2010). Canine placenta: A source of prepartal prostaglandins during normal and antiprogestin-induced

parturition. *Reproduction*, *139*(3), 655–664. https://doi.org/10.1530/REP-09-0140

Kowalewski, M. P., Gram, A., Kautz, E., & Graubner, F. R. (2015). The dog: Nonconformist, not only in maternal recognition signaling. *Advances in Anatomy, Embryology and Cell Biology.*, *216*, 215–237.

Kowalewski, M. P., Kazemian, A., Klisch, K., Gysin, T., Tavares Pereira, M., & Gram, A. (2021). Canine endotheliochorial placenta: Morpho-functional aspects. *Advances in Anatomy, Embryology, and Cell Biology*, *234*, 155–179. https://doi.org/10.1007/978-3-030-77360-1_8

Kowalewski, M. P., Meyer, A., Hoffmann, B., Aslan, S., & Boos, A. (2011). Expression and functional implications of peroxisome proliferator-activated receptor gamma (PPARgamma) in canine reproductive tissues during normal pregnancy and parturition and at antiprogestin induced abortion. *Theriogenology*, *75*(5), 877–886. https://doi.org/10.1016/j.theriogenology.2010.10.030

Kowalewski, M. P., Michel, E., Gram, A., Boos, A., Guscetti, F., Hoffmann, B., Aslan, S., & Reichler, I. (2011). Luteal and placental function in the bitch: Spatio-temporal changes in prolactin receptor (PRLr) expression at dioestrus, pregnancy and normal and induced parturition. *Reproductive Biology and Endocrinology*, *9*, 109. https://doi.org/10.1186/1477-7827-9-109

Kowalewski, M. P., Tavares Pereira, M., & Kazemian, A. (2020). Canine conceptus-maternal communication during maintenance and termination of pregnancy, including the role of species-specific decidualization. *Theriogenology*, *150*, 329–338. https://doi.org/10.1016/j.theriogenology.2020.01.082

Kowalewski, M. P., Tavares Pereira, M. T., Papa, P., & Gram, A. (2020). Progesterone receptor blockers: Historical perspective, mode of function and insights into clinical and scientific applications. *Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere*, *48*(6), 433–440. https://doi.org/10.1055/a-1274-9290

Kreis, N. N., Louwen, F., & Yuan, J. (2019). The multifaceted p21 (Cip1/Waf1/CDKN1A) in cell differentiation, migration and cancer therapy. *Cancers (Basel)*, *11*(9), 1220. https://doi.org/10.3390/cancers11091220

Lee, K. Y., Jeong, J. W., Wang, J., Ma, L., Martin, J. F., Tsai, S. Y., Lydon, J. P., & DeMayo, F. J. (2007). Bmp2 is critical for the murine uterine decidual response. *Molecular and Cellular Biology*, *27*(15), 5468–5478. https://doi.org/10.1128/MCB.00342-07

Leonhardt, S. A., & Edwards, D. P. (2002). Mechanism of action of progesterone antagonists. *Experimental Biology and Medicine (Maywood, N.J.)*, *227*(11), 969–980.

Lok, C. A., Nieuwland, R., Sturk, A., Hau, C. M., Boer, K., Vanbavel, E., & Vanderpost, J. A. (2007). Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets*, *18*(1), 68–72. https://doi.org/10.1080/09537100600864285

Maroni, D., & Davis, J. S. (2011). TGFB1 disrupts the angiogenic potential of microvascular endothelial cells of the corpus luteum. *Journal of Cell Science*, *124*(Pt 14), 2501–2510. https://doi.org/10.1242/jcs.084558

Martin, N., Hoftmann, T., Politt, E., Hoppen, H. O., Sohr, M., Gunzel-Apel, A. R., & Einspanier, A. (2009). Morphological examination of the corpora lutea from pregnant bitches treated with different abortifacient regimes. *Reproduction in Domestic Animals*, *44*(Suppl 2), 185–189. https://doi.org/10.1111/j.1439-0531.2009.01430.x

Massague, J. (2012). TGFbeta signalling in context. *Nature Reviews. Molecular Cell Biology*, *13*(10), 616–630. https://doi.org/10.1038/nrm3434

Maynard, S. E., Min, J.-Y., Merchan, J., Lim, K.-H., Li, J., Mondal, S., Libermann, T. A., Morgan, J. P., Sellke, F. W., Stillman, I. E., Epstein, F. H., Sukhatme, V. P., & Karumanchi, S. A. (2003). Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of Clinical Investigation*, *111*(5), 649–658. https://doi.org/10.1172/jci17189

McElrath, T. F., Cantonwine, D. E., Gray, K. J., Mirzakhani, H., Doss, R. C., Khaja, N., Khalid, M., Page, G., Brohman, B., Zhang, Z., Sarracino, D., & Rosenblatt, K. P. (2020). Late first trimester circulating microparticle proteins predict the risk of preeclampsia <35 weeks and suggest phenotypic differences among affected cases. *Scientific Reports*, *10*(1), 17353. https://doi.org/10.1038/s41598-020-74078-w

Michel, J. J., & Scott, J. D. (2002). AKAP mediated signal transduction. *Annual Review of Pharmacology and Toxicology*, *42*, 235–257. https://doi.org/10.1146/annurev.pharmtox.42.083101.135801

Mistry, H. D., Ogalde, M. V. H., Broughton Pipkin, F., Escher, G., & Kurlak, L. O. (2020). Maternal, fetal, and placental selectins in women with pre-eclampsia; Association with the Renin-Angiotensin-System. *Frontiers in Medicine*, *7*, 270. https://doi.org/10.3389/fmed.2020.00270

Monsivais, D., Clementi, C., Peng, J., Fullerton, P. T., Jr., Prunskaite-Hyyrylainen, R., Vainio, S. J., & Matzuk, M. M. (2017). BMP7 induces uterine receptivity and blastocyst attachment. *Endocrinology*, *158*(4), 979–992. https://doi.org/10.1210/en.2016-1629

Nelson, P. J., Moissoglu, K., Vargas, J., Jr., Klotman, P. E., & Gelman, I. H. (1999). Involvement of the protein kinase C substrate, SSeCKS, in the Actin-based stellate morphology of mesangial cells. *Journal of Cell Science*, *112*(Pt 3), 361–370. https://doi.org/10.1242/jcs.112.3.361

Nohr, B., Hoffmann, B., & Steinetz, B. E. (1993). Investigation of the endocrine control of parturition in the dog by application of an antigestagen. *Journal of Reproduction and Fertility. Supplement*, *47*, 542–543.

Nowak, M., Gram, A., Boos, A., Aslan, S., Ay, S. S., Onyay, F., & Kowalewski, M. P. (2017). Functional implications of the utero-placental relaxin (RLN) system in the dog throughout pregnancy and at term. *Reproduction*, *154*(4), 415–431. https://doi.org/10.1530/REP-17-0135

Nowak, M., Rehrauer, H., Ay, S. S., Findik, M., Boos, A., Kautz, E., & Kowalewski, M. P. (2019). Gene expression profiling of the canine placenta during normal and antigestagen-induced luteolysis. *General and Comparative Endocrinology*, *282*, 113194. https://doi.org/10.1016/j.ygcen.2019.05.019

Ojasoo, T., Dore, J. C., Gilbert, J., & Raynaud, J. P. (1988). Binding of steroids to the progestin and glucocorticoid receptors analyzed by correspondence analysis. *Journal of Medicinal Chemistry*, *31*(6), 1160–1169. https://doi.org/10.1021/jm00401a015

Palmer, K. R., Tong, S., & Kaitu'u-Lino, T. J. (2017). Placental-specific sFLT-1: Role in pre-eclamptic pathophysiology and its translational possibilities for clinical prediction and diagnosis. *Molecular Human Reproduction*, *23*(2), 69–78. https://doi.org/10.1093/molehr/gaw077

Papa, P. C., & Kowalewski, M. P. (2020). Factors affecting the fate of the canine corpus luteum: Potential contributors to pregnancy and non-pregnancy. *Theriogenology*, *150*, 339–346. https://doi.org/10.1016/j.theriogenology.2020.01.081

Phipps, E. A., Thadhani, R., Benzing, T., & Karumanchi, S. A. (2019). Pre-eclampsia: Pathogenesis, novel diagnostics and therapies. *Nature Reviews. Nephrology*, *15*(5), 275–289. https://doi.org/10.1038/s41581-019-0119-6

Polisca, A., Scotti, L., Orlandi, R., Brecchia, G., Maranesi, M., Zerani, M., & Boiti, C. (2010). Aglepristone (RU534) administration to non-pregnant bitches in the mid-luteal phase induces early luteal regression. *Theriogenology*, *74*(4), 672–681. https://doi.org/10.1016/j.theriogenology.2010.03.021

Schneider, M. P., Boesen, E. I., & Pollock, D. M. (2007). Contrasting actions of endothelin ET(a) and ET(B) receptors in cardiovascular disease. *Annual Review of Pharmacology and Toxicology*, *47*, 731–759. https://doi.org/10.1146/annurev.pharmtox.47.120505.105134

Sircar, M., Thadhani, R., & Karumanchi, S. A. (2015). Pathogenesis of pre-eclampsia. *Current Opinion in Nephrology and Hypertension*, *24*(2), 131–138. https://doi.org/10.1097/MNH.0000000000000105

Tamura, I., Asada, H., Maekawa, R., Tanabe, M., Lee, L., Taketani, T., Yamagata, Y., Tamura, H., & Sugino, N. (2012). Induction of IGFBP-1 expression by cAMP is associated with histone acetylation status of the promoter region in human endometrial stromal cells. *Endocrinology*, *153*(11), 5612–5621. https://doi.org/10.1210/en.2012-1420

Tavares Pereira, M., Kazemian, A., Rehrauer, H., & Kowalewski, M. P. (2022). Transcriptomic profiling of canine decidualization and effects of antigestagens on decidualized dog uterine stromal cells. *Scientific Reports*, *12*(1), 21890. https://doi.org/10.1038/s41598-022-24790-6

Tavares Pereira, M., Nowaczyk, R., Aslan, S., Ay, S. S., & Kowalewski, M. P. (2021). Utero-placental immune milieu during normal and aglepristone-induced parturition in the dog. *Animals (Basel)*, *11*(12), 3598. https://doi.org/10.3390/ani11123598

Tavares Pereira, M., Nowaczyk, R., Payan Carreira, R., Miranda, S., Aslan, S., Kaya, D., & Kowalewski, M. P. (2021). Selected uterine immune events associated with the establishment of pregnancy in the dog. *Frontiers in Veterinary Science*, *9*(7), 625921. https://doi.org/10.3389/fvets.2020.625921

Tavares Pereira, M., Schuler, G., Aslan, S., Payan-Carreira, R., Reichler, I. M., Reynaud, K., & Kowalewski, M. P. (2023). Utero-placental expression and functional implications of HSD11B1 and HSD11B2 in canine pregnancy. *Biology of Reproduction*, *108*, 645–658. https://doi.org/10.1093/biolre/ioac214

Telgmann, R., & Gellersen, B. (1998). Marker genes of decidualization: Activation of the decidual prolactin gene. *Human Reproduction Update*, *4*(5), 472–479.

Telgmann, R., Maronde, E., Tasken, K., & Gellersen, B. (1997). Activated protein kinase a is required for differentiation-dependent transcription of the decidual prolactin gene in human endometrial stromal cells. *Endocrinology*, *138*(3), 929–937. https://doi.org/10.1210/endo.138.3.5004

Ulu, I., Cekmez, Y., Yildirim Kopuk, S., Ozer, N., Yogurtcuoglu, E. E., Angin, P., & Kiran, G. (2019). Maternal serum thrombospondin-1 is significantly altered in cases with established preeclampsia. *The Journal of Maternal-Fetal & Neonatal Medicine*, *32*(15), 2543–2546. https://doi.org/10.1080/14767058.2018.1441279

Vermeirsch, H., Simoens, P., Hellemans, A., Coryn, M., & Lauwers, H. (2000). Immunohistochemical detection of progesterone receptors in the canine uterus and their relation to sex steroid hormone levels. *Theriogenology*, *53*(3), 773–788. https://doi.org/10.1016/S0093-691X(99)00273-3

Vermeirsch, H., Simoens, P., & Lauwers, H. (2000). Immunohistochemical detection of the estrogen receptor-alpha and progesterone receptor in the canine pregnant uterus and placental labyrinth. *The Anatomical Record*, *260*(1), 42–50.

Wanke, M., Loza, M. E., Monachesi, N., & Concannon, P. (1997). Clinical use of dexamethasone for termination of unwanted pregnancy in dogs. *Journal of Reproduction and Fertility. Supplement*, *51*, 233–238.

Woodward, D. F., Jones, R. L., & Narumiya, S. (2011). International Union of Basic and Clinical Pharmacology. LXXXIII: Classification of prostanoid receptors, updating 15 years of progress. *Pharmacological Reviews*, *63*(3), 471–538. https://doi.org/10.1124/pr.110.003517

Zatta, S., Rehrauer, H., Gram, A., Boos, A., & Kowalewski, M. P. (2017). Transcriptome analysis reveals differences in mechanisms regulating cessation of luteal function in pregnant and non-pregnant dogs. *BMC Genomics*, *18*(1), 757. https://doi.org/10.1186/s12864-017-4084-9

Zenclussen, A. C., Fest, S., Sehmsdorf, U. S., Hagen, E., Klapp, B. F., & Arck, P. C. (2001). Upregulation of decidual P-selectin expression is associated with an increased number of Th1 cell populations in patients suffering from spontaneous abortions. *Cellular Immunology*, *213*(2), 94–103. https://doi.org/10.1006/cimm.2001.1877

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