



## Early development of the corpus luteum in mares

*O processo inflamatório na formação do corpo luteo da égua*

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**Keywords:** corpus luteum, cytokines, equine, inflammation, luteinization.

**Palavras-chave:** citocina, corpo luteo, equino, inflamação, luteinização.

### The mature follicle

In a manuscript published in 1897, the pioneer of the embryo transfer procedure, Walter Heape, ponders on the findings of Leopold and Mironoff (1894) who described human follicles approaching ovulation were characterized by a marked congestion and “chronic inflammation”. It would be almost 100 years later that Lawrence Espey proposed the hypothesis that ovulation is comparable to an inflammation process. In that work, the ovulatory process was compared to an inflammation-like reaction owing to similar events triggered in the follicle micro-anatomy by the LH-surge: hyperemia, congestion, increased vascular permeability, and the recruitment and influx of leukocytes into the follicle wall and regional ovarian tissue (Espey, 1980, 1994).

In mares, a well-defined LH surge does not occur as it does in most domestic species; instead a steady increase in LH concentrations lead to oocyte maturation and follicular rupture with LH concentrations actually peaking ~ a day after ovulation. Kerban et al. (1999) described inflammation-like changes that also occur in the preovulatory follicle of mares after treatment with human chorionic gonadotropin (hCG). These changes were more pronounced after 24 h post administration of hCG, including severe edema, hyperemia and hemorrhage in the preovulatory follicle. At 33 h post hCG, there is maximal expression of the enzyme cyclooxygenase-2, which precedes increasing intrafollicular concentrations of PGE2 and PGF2 $\alpha$ . In addition, hCG treatment resulted in an up-regulation of mRNA and protein for receptors of prostaglandin E2 in granulosa cells at 12 and 39 h post-hCG that coincides with increased preovulatory follicular PGE2 synthesis, an important agent that mediates ovulation.

Therefore, events that resemble an inflammatory reaction have been also confirmed to occur in horses. Follicular rupture, the discharge of the oocyte and initial stages of transformation of follicle cells into luteal cells occur in an “inflamed” environment. These cellular changes continue to evolve as ovulation takes place and early luteal development begins.

### Early luteinization

The differentiation of follicular cells (theca interna and granulosa) into luteal cells is characterized by cessation of proliferation of these cells, accompanied by marked hypertrophy of the granulosa cells. In horses, the theca interna cells appear to degenerate just before ovulation and therefore, assumed to not contribute to the formation of the corpus luteum. It remains, however, to be determined the mechanism involved in this phenomenon, that is, whether they degenerate by necrosis or apoptosis.

It is evident that preovulatory luteinization takes place soon after hCG stimulation. Although not reflected in concentrations of estradiol and progesterone in the peripheral blood, intrafollicular concentrations of these hormones attest to a marked switch from estrogen to progesterone production most evident after 24 h following hCG treatment (Pinto et al., 2003). Soon after ovulation, granulosa cells rapidly undergo hypertrophy denoted by an increase in cell diameter from 10  $\mu$ m to ~40  $\mu$ m. The ability of the corpus luteum to secrete progesterone thus increases quantitatively and qualitatively with concentrations of progesterone rapidly approaching double digits by 5 or 6 days after ovulation in most mares (Barker et al., 2006).

### Angiogenesis

During early luteal development, the vast majority of proliferating cells are endothelial cells that support the dramatic angiogenesis that occurs in the corpus luteum. In addition, angiogenic cytokines such as fibroblast growth factor, vascular endothelial growth factor, platelet-derived growth factor and tumor necrosis factor work in concert to modulate the extensive and rapid angiogenic processes that take place in the early developing corpus luteum. These processes and related angiogenic factors seem to share similarities among several species, including the horse. Moreover, nitric oxide seems to exert angiogenic activity in the equine CL, likely via its effects on proliferation of endothelial cells and secretion of VEGF. Angiogenesis is essential for luteal function. In the rat, it has been shown that corpora lutea consume two to six times more oxygen per unit weight than does other vital organs, such as the liver, kidney or heart (Swann and Bruce, 1987).



### *Polymorphonuclear Leukocytes (PML)*

Not surprisingly, white blood cells, similar to their presence in preovulatory follicles, have also been shown to be present in the early developing corpus luteum. In the bovine corpus luteum, there is a rapid increase in neutrophils, eosinophils and macrophages during early luteal development. These leukocytes obviously contribute to the presence of innate immunity within the corpus luteum but also seem to synergistically participate in luteal angiogenesis. In fact, interleukin-8 (IL-8), an important angiogenic factor, has been shown to chemoattract the migration of PML. Macrophages and eosinophils have also been implicated in CL development and angiogenesis; their migration into the development CL of non-pregnant and pregnant cows is well defined. Conditions leading to leukopenia may affect CL function and luteal angiogenesis. Using a mouse model to conditionally ablate macrophage by targeted toxicity with diphtheria toxin, it was shown that macrophage ablation led to disruption of the ovarian vasculature, resulting in intraovarian hemorrhage and necrosis (Turner, et al., 2011).

### *Prostaglandins*

In a study using a microdialysis system to culture luteal cells from bovine developing CL (1- to 4 days), fibroblast growth factor and vascular endothelial growth factor were shown to upregulate the secretion of PGF<sub>2α</sub> that, during early CL development appears to favor luteal function (Kobayashi et al., 2001). These authors argue for a dual role for PGF<sub>2α</sub>: anti-luteolytic and stimulator of angiogenic factors during early luteal development and luteolytic during midcycle. Thus, both PGEs and PGF<sub>2α</sub> work as luteotrophic agents during early development of the corpus luteum.

### **Summary**

Events leading to maturation of the equine preovulatory and ovulation resemble those present in an inflammatory reaction. Once ovulation takes place, luteinizing granulosa cells quickly continue to undergo functional and structural changes that results in a remarkable growth of the luteal gland and increasing output of progesterone. The developing corpus luteum requires the establishment of a robust development of extracellular matrix and vascular network (angiogenesis). This development and regulation is governed by several growth factors, cytokines, polymorphonuclear leukocytes, and prostaglandins that work in and autocrine and paracrine to achieve successful luteal function, especially if pregnancy is taking place.

Most of the information available regarding early development of the corpus luteum and its regulation derive from studies in humans, ruminants and laboratory animals. Recently, considerable attention has been given to elucidating these events in the equine corpus luteum. While much of the available research conducted in other species can be extrapolated to equine species, it is critical that we continue to learn more about the intrinsic peculiarities about the CL formation and regulation in horses.

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