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The equine cyclic corpus luteum: microvascularization, luteal cells characterization and function

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Summary

Early embryonic mortality in the mare is frequently associated with a deficient progesterone (P4) production. Ovarian structures dysfunction might be related to a deficient vascularization, since corpus luteum (CL) formation and its endocrine function are dependent on capillary growth. Therefore, in order to understand deficient equine luteal function, the present overview aimed at eventual changes in angiogenic activity of cyclic luteal structures and their relationship to luteal endocrinological function and luteal cell dynamics. These studies were performed on luteal tissue collected post mortem from cycling mares. Based on morphological structure and plasma P4 values, luteal tissue was classified. On luteal tissue sections blood vessels were marked by histochemical techniques. Microvascular density, number and size of luteal cells were determined with a computerized image analysis system. Progesterone receptors in luteal tissue were detected by immunocytochemistry. Vascular density did not differ for any luteal structures. Large luteal cells number in the corpus hemorraghicum (CH) was lower than in other structures ($p < 0.05$). Small luteal cells number and size did not differ. Only large luteal cells stained positively for P4 receptors. Plasma P4 was increased in the mid and late luteal phases, when compared to both follicular and corpus hemorrhagicum phases ($p £ 0.05$). These findings suggest that vascular growth of cyclic luteal structures in the mare is coordinated with the development of non vascular tissue, enabling P4 synthesis to start early in the luteal phase. This hormone is most likely synthesized by large luteal cells, showing a direct relationship between the number of these cells and plasma P4 levels.

Keywords: Mare, corpus luteum, microvascularization, luteal cells, progesterone receptors.

Das equine zyklische Corpus luteum: Mikrovaskularisation, Funktion und Charakterisierung der Luteinzellen

Die frühembryonale Mortalität bei Stuten ist häufig mit einer verminderten Progesteronproduktion (P4) verbunden. Dysfunktionen ovarieller Strukturen sind möglicherweise auf eine ungenügende Vaskularisation zurückzuführen, da die Entstehung eines Corpus luteum (CL) und dessen endokrine Funktion von der kapillaren Entwicklung abhängen. Ziel dieser Studie war es, mögliche Veränderungen in der Aktivität der Angiogenese in zyklischen Gelbkörpern aufzuzeigen und ihre Beziehung zur endokrinen Funktionen und der Zelldynamik des Gelbkörpers darzustellen. Als Untersuchungsgut diente post mortem entnommenes Gelbkörpergewebe zyklischer Stuten, welches anhand morphologischer Strukturen und der Plasma-P4 Konzentrationen klassifiziert wurde. Die lutealen Blutgefäße wurden im histologischen Schnitt mittels histochemischer, die Progesteronrezeptoren mittels immunhistochemischer Nachweisverfahren dargestellt. Die mikrovaskuläre Dichte sowie die Anzahl und Größe der Luteinzellen wurde durch ein computergestütztes Bildanalysesystem bestimmt. Innerhalb der morphologischen Strukturen der verschiedenen Gelbkörper konnten bezüglich der Gefäßdichte keine Unterschiede aufgezeigt werden. Die Anzahl großer Luteinzellen war im Corpus hämorrhagicum (CH) geringer als in den anderen Gruppen ($p < 0,05$), wohingegen die Anzahl und Größe der kleinen Luteinzellen nicht variierte. Lediglich in den großen Luteinzellen konnten P4-Rezeptoren nachgewiesen werden. Im Vergleich zur mittleren und späten Gelbkörperphase war die Plasma- P4 Konzentration sowohl in der Follikel- als auch in der CH-Phase erhöht ($p £ 0,05$). Diese Ergebnisse weisen darauf hin, dass das Gefäßwachstum und die Entwicklung von nicht vaskularisiertem Gewebe innerhalb des zyklischen Gelbkörpergewebes der Stute in einem direktem Zusammenhang stehen, wodurch eine P4 Synthese in der frühen Gelbkörperphase ermöglicht wird. Es ist sehr wahrscheinlich, dass dieses Hormon von den großen Luteinzellen synthetisiert wird, da die Anzahl dieser Zellen und die nachgewiesene Plasma-P4 Konzentration in einer direkten Beziehung zueinander stehen.

Schlüsselwörter: Reproduktion, Stute, Corpus luteum, Mikrovaskularisation, Luteinzellen, Progesteronrezeptor

Introduction

After ovulation, the corpus luteum (CL) forms from the ovulatory follicle as a transient endocrine gland (Niswender and Nett., 1988). During the ovarian/uterine cycle, luteal structures exhibit a physiologic growth and regression accompanied by fast changes in angiogenesis and blood flow (*Redmer* and *Reynolds* 1996, *Modlich* et al. 1996).

Early embryonic mortality in the mare is frequently associated with a deficient progesterone (P4) production. Ovarian structures dysfunction might be related to a deficient vascularization, since the CL formation and its endocrine function are closely dependent on the growth of new capillaries (*Redmer* et al., 1988). Such situation might eventually result in a decrease in P4 secretion, which is responsible for an adverse uterine environment for the conceptus and consequently early embryonic death and infertility. Also, angiopathies in the mare´s reproductive tract have been associated with infertility (*Schoon* et al. 1997). In the mare, most pregnancy losses occur before day 40 of gestation when the CL is the only source of P4 (*Allen* 2001), suggesting embryonic mortality might be associated with a primary luteal insufficiency. Therefore, in order to understand deficient luteal function, the present overview aimed at the angiogenic activity of cyclic luteal structures and their relationship to luteal endocrinological function and luteal cell type dynamics in the mare.

Vascular Growth in Ovarian tissues

Some organs of the female reproductive tract, namely the ovary, exhibit a periodic and fast physiologic development and regression process, with simultaneous fast changes in

blood flow (Reynolds et al. 1992). This tissue formation and regression appears to be stimulated by angiogenic substances and inhibited by anti-angiogenic factors (*Folkman* and *Klagsburn* 1987, *Redmer* et al. 1991). In the mare, recent studies on microvascularization of the reproductive tract such as the endometrium, follicles and corpus luteum have been referred (*Al-zi´abi* et al. 2000, *Ferreira-Dias* et al. 2001a, *Watson* and *Ai-zi´abi* 2002). Knowledge of angiogenesis might help understand infertility in the mare due to primary luteal function impairment.
In diestrus, blood flow in the equine ovary suffers characteristic changes throughout the oestrous cycle, with an indirect relationship between resistance to ovarian blood flow and plasma P4 levels (*Bollwein* et al. 2002a). Blood flow in the mare´s CL depends mainly on the day of the oestrous cycle reaching its maximum on day 5 after ovulation and decreasing by the middle of diestrus, some days before the decrease of P4 is detected in blood (*Bollwein* et al. 2002b).
Antigenic activity of cow CL increases with age of the luteal structure (*Redmer* et. al. 1988). However, in another study, dramatic changes in bovine luteal vascularization were in agreement with cyclic tissue development and regression, increasing from early to middle stage and falling to late stage (*Zheng* et al. 1993).

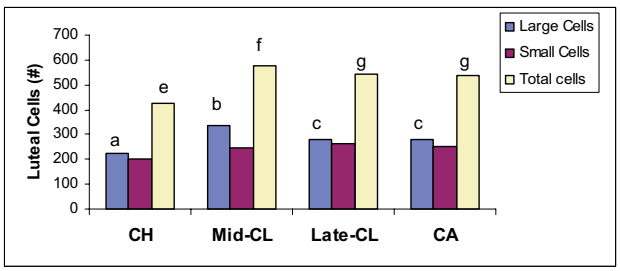
In a preliminary study performed in our laboratory, luteal tissue and blood were collected during the breeding season at an abattoir from randomly assigned cycling mares. Based on morphological structure and plasma P4 values, luteal tissue was classified as corpus hemorraghicum (CH), mid luteal phase CL (mid-CL), late or regressing CL (late-CL) and non functional CL (corpus albicans-CA). Luteal tissue was fixed in carnoy solution for histology. Blood vessels were marked on histologic sections by histochemical techniques, using periodic acid-Schiff stain. Vascular density and number and size of luteal cells were determined with a computerized image analysis system (CAS, Beckton Dickinson) based on the percentage of total histologic area occupied by vascular lumen. Vascular density did not differ for any luteal structures evaluated. There was an increase in plasma P4 in mid and late luteal phases when compared to both follicular and corpus hemorraghicum phases (p £ 0.05) (*Ferreira-Dias* et al. 2001b). These findings might suggest that vascular growth of cyclic luteal structures in the mare is coordinated with the development of non vascular tissue, enabling P4 synthesis to start at a very early stage of the luteal structure (*Ferreira-Dias* et al. 2001b).

Luteal Cell Dynamics

The histologic observation of luteal structures has shown that this tissue is formed by small and large luteal cells and non luteal cells, such as fibroblasts and macrophages (*Lei* et al. 1991, *Broadley* et al. 1994), and by blood vessels that rapidly develop after ovulation and regress during luteolysis (*Modlich* et al. 1996). These two different luteal cell types can be distinguished on the basis of morphological and biochemical criteria (*Lei* et al. 1991). These cell populations, with distinct functional characteristics, are present in the pig (*Richards* et al. 1994), cow (*Lei* et al., 1991), mare (*Broadley* et al. 1994), woman (*Lei* et al. 991) and sheep corpus luteum (*Fitz* et al. 1982).

Recently, another study on histologic sections of equine luteal tissue, confirmed the existence of large and small luteal cells in all cyclic luteal structures (CH, mid-CL, late-CL and CA)(*Ferreira-Dias* et al. 2002). The number of large luteal cells in the CH was lower than in other structures (P<0.05)(*Ferreira-Dias* et al. 2002). The mean number of this type of cell in the mid-CL was higher than in the CH, late-CL and CA (p £ 0,05)(Fig.1). Since P4 production followed the same pattern of large luteal cells, this suggests that large luteal cells might play an important role on this hormone synthesis in the mare. However, no significant difference was observed between the amount of large luteal cells in the late-

Fig 1 Quantification of large and small luteal cells in different luteal structures. CH = corpus hemorraghicum; Mid-CL = mid luteal phase corpus luteum; late-CL = late or regressing CL; CA= corpus albicans. Columns with different superscripts differ significantly (p<0.05).
Quantifizierung der großen und kleinen Luteinzellen in unterschiedlichen Differenzierungsformen von Gelbkörpern. CH = Corpus hämorrhagicum, Mid-CL = mittlere Gelbkörperphase, late-CL = späte Gelbkörperphase oder Corpus luteum in Regression, CA = Corpus albicans. Säulen mit unterschiedlichen Überschriften unterscheiden sich signifikant (p<0,05).



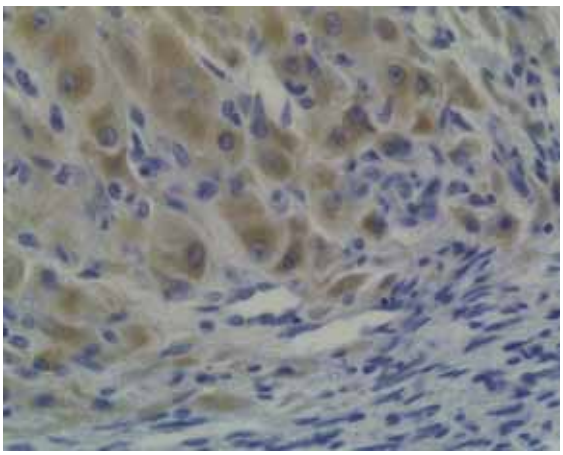
CL and in the CA (Fig.1), which seems contradictory. Nevertheless, in spite of the high number of large luteal cells in the CA, these cells showed signs of cytoplasmatic degeneration, such as vacuoles and fragmentation. Their nucleus presented karyorrhexis or pyknosis, both indicators of cell destruction. All these aspects fully justify the inability of P4 synthesis by this non functional luteal tissue.
When the number of small luteal cells was compared in different luteal structures, no difference was observed (p £ 0,05)(Fig.1). The total number of large and small luteal cells was reduced in the CH when compared to mid- and late-CL (p £ 0,05), but no significant difference was observed among the remaining luteal structures (Fig.1). Besides, the size of either large or small luteal cells did not change when all the luteal structures were compared (p £ 0.05)(*Ferreira-Dias* et al. 2002).

Luteal Cell Function - Progesterone Production

Even though in sex-steroid sensitive tissues P4 decreases the expression of progesterone receptors (PR), in steroidogenic tissue such as the CL this might be different (*Ottander* et al. 2000). Progesterone receptors have been referred in the canine ovary (*Vermeirsch* et al. 2001), bovine CL (*Rueda* et al. 2000) and porcine CL (*Slomczynska* et al. 2000).
A stimulatory role of PR-mediated action in the steroidogenic cells of human and bovine CL has been referred (*Rueda* et al. 2000; *Ottander* et al. 2000). It appears there is an autocrine/paracrine role of steroid receptors in the regulation of corpus luteum genesis and function (*Rueda* et al. 2000). This

hormone also represses the onset of apoptosis in the CL by a PR-dependent mechanism (*Rueda* et al. 2000). In the mare, both large and small dissociated luteal cells were capable of in vitro production of P4 (*Broadley* et al. 1994). However, immunocytochemistry assays revealed that, in the mare, only large luteal cells showed positive staining for P4 receptors (Fig. 2)(*Ferreira-Dias* et al. 2002). Staining intensity increased from CH to mid-CL, and decreased from late-CL to CA ($p<0.05$). Immunocytochemistry assays suggest that, in the mare, large luteal cells may play a major role on P4 production and maintenance of luteal function.

Fig 2 Immunolocalization of progesterone receptors in the equine CL. Only large luteal cells stained positively
Immunhistochemischer Nachweis des Progesteron-Rezeptors im equinen CL. Positiver Nachweis nur in großen Luteinzellen



Conclusions

In conclusion, the results obtained confirm that the endocrine activity of luteinized tissues of the equine ovary starts at a very early stage, right after ovulation. This functional capability of the mare CL may be dependent on vascular growth and regression of cyclic luteal structures which might be coordinated with the development of non vascular tissue. A direct relationship between plasmatic values of P4 and the number of large luteal cells and P4 receptors suggests that these cells might play an important role on the synthesis of this hormone as well as maintenance of luteal function.

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