Characterization of cellular and vascular changes in equine follicles during hCG-induced ovulation

A. Kerban¹, M. Doré² and J. Sirois^{1*}

¹Centre de Recherche en Reproduction Animale and ²Département de Pathologie et Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6

In contrast to other species, the histology of the equine follicle during ovulation has not been described. Preovulatory follicles were isolated during oestrus at 0, 12, 24, 30, 33, 36 and 39 h ($n = 5\text{--}6$ follicles per time point) after an ovulatory dose of hCG to characterize the cellular and vascular changes associated with ovulation in mares. Pieces of follicle wall were formalin-fixed and processed for light microscopy to evaluate the general follicular morphology and quantify selected parameters. Marked changes were observed in the histology of equine follicles in the hours before ovulation. The thickness of the granulosa cell layer doubled between 0 and 39 h after hCG (77.8 ± 4.8 versus 158.8 ± 4.8 μm , respectively; $P < 0.01$). This expansion was caused primarily by a pronounced accumulation of acid mucosubstances between granulosa cells, which was first detected at 12 h after hCG and peaked at 36–39 h. In contrast, a significant thinning of the theca interna was observed after hCG treatment. Fewer cell layers were present; theca interna cells appeared smaller than before hCG; and the presence of occasional pyknotic cells was noted at 36 and 39 h after hCG. In addition, the theca layers were invaded by numerous eosinophils. No eosinophils were observed in preovulatory follicles isolated between 0 and 24 h after hCG, but the number increased to 14.0 ± 0.8 and 5.6 ± 0.3 eosinophils per field ($\times 400$) in theca interna and theca externa, respectively, 39 h after hCG treatment ($P < 0.01$). Severe oedema, hyperaemia and haemorrhages, and significant increases in the number of blood vessels in theca interna and externa were observed at 33, 36 and 39 h after hCG. This study provides the first in-depth characterization of the sequential cellular and vascular changes that occur in equine follicles before ovulation.

Introduction

Ovarian follicular development and ovulation in mares has some unique characteristics compared with that of other large animals. Mares are seasonal polyoestrous animals; the duration of the oestrous cycle is about 20–21 days and is characterized by a relatively long period of oestrus (follicular phase of 5–7 days) (Palmer, 1978; Ginther, 1992; Daels and Hughes, 1993). In most cases, a single cohort or wave of follicles larger than 15 mm in diameter develops during the oestrous cycle, and the preovulatory follicle is recruited 13–14 days before ovulation (Driancourt and Palmer, 1984; Pierson and Ginther, 1987; Sirois *et al.*, 1989). The maximal size of the preovulatory follicle (40–45 mm in diameter) is considerably larger than in other species. Ovulation in mares is not triggered by a typical LH surge. Instead, there is a progressive increase in LH during oestrus, which typically peaks 1 day after ovulation (Whitmore *et al.*, 1973; Stabenfeldt *et al.*, 1975; Alexander and Irvine, 1982), indicating that the ovulatory cascade in horses is initiated when a threshold concentration of gonadotrophin is reached. However, the

hormonal and cellular control of oestradiol biosynthesis in the developing equine preovulatory follicle appears to follow the two-cell (theca interna and granulosa cells), two-gonadotrophin (LH and FSH) model that is common to other species (Sirois *et al.*, 1991; Almadhidi *et al.*, 1995).

Ovulation in mares can be induced during the follicular phase by the administration of hCG, and the duration of the ovulation, defined as the interval from hCG injection to follicular rupture, is approximately 36–48 h (Duchamp *et al.*, 1987; Watson *et al.*, 1991). In contrast to other species in which follicular rupture occurs over the majority of the ovarian surface, the equine ovulatory process is restricted to a specific region of the ovary, the ovulatory fossa (Stabenfeldt *et al.*, 1975). Some of the biophysical characteristics of equine ovulation have been described using transrectal palpation and ultrasonography. As in other species, rupture does not appear to result from a build-up of pressure within the follicle (Ginther, 1992). The shape and consistency of the equine follicle change as ovulation approaches, from a relatively firm spherical structure to a soft non-spherical (pear-shaped or conical) structure (Pierson and Ginther, 1985). Two patterns of follicular fluid evacuation have been observed after rupture: an abrupt process in which 90% of the fluid is evacuated during the first 60 s, and a more

*Correspondence.

Received 13 November 1998.

gradual process in which complete evacuation requires 6–7 min (Townson and Ginther, 1987, 1989).

Elucidating the complex cascade of cellular and biochemical events leading to follicular rupture has been the focus of many studies, and the subject of several reviews (Murdoch and Cavender, 1987; Thibault and Levasseur, 1988; Goetz *et al.*, 1991; Tsafiri *et al.*, 1993; Espey and Lipner, 1994). Descriptive histological studies of ovulation have been performed in numerous species, including rabbits (Espey, 1967; Bjersing and Cajander, 1974), various rodents (Byskov, 1969; Parr, 1974; Martin and Talbot, 1987), sheep (Cavender and Murdoch, 1988), cattle (Priedkalns *et al.*, 1968) and humans (Okamura *et al.*, 1980). Whereas differences were noted among species, the collective findings revealed a clear parallel between the gonadotrophin-dependent process and a classic inflammatory reaction (Espey, 1980; Espey and Lipner, 1994). As observed in acute inflammation, hyperaemia, congestion, increased vascular permeability, and the recruitment of white blood cells are present during ovulation. The sequential histology of ovulation has not been characterized in mares, although the microscopic anatomy of the corpus luteum has been described (Harrison, 1946; Van Niekerk *et al.*, 1975; Levine *et al.*, 1979). The objective of this study was to characterize the cellular and vascular changes occurring during the final differentiation of equine preovulatory follicles after a gonadotrophin-induced ovulation to understand better the precise sequence of events leading to follicular rupture in horses.

Materials and Methods

Materials

Lutalyse was purchased from UpJohn (Kalamazoo, MI) and hCG was obtained from The Buttlar Company (Columbus, OH). Rompun was purchased from Haver (Bayvet Division, Shawnee, KS). Torbugesic was obtained from Fort Dodge Laboratories Inc. (Fort Dodge, IA). Dormosedan was purchased from SmithKline Beecham, Animal Health (West Chester, PA). Culture medium was obtained from Gibco Bethesda Research Laboratories Life Technologies Inc. (Gaithersburg, MD).

Animals

Adult Standardbred and Thoroughbred mares, weighing approximately 375–450 kg, were kept outdoors and fed once a day with hay and concentrates. They were teased once a day with a pony stallion for detection of oestrus, and ovarian follicular development was monitored once a day by transrectal real-time ultrasonography (Sirois *et al.*, 1989). When the preovulatory follicle reached 35 mm in diameter during oestrus, the animal was injected with 2500 iu hCG i.v. to induce ovulation.

Ovariectomy and isolation of preovulatory follicles

The ovary bearing the presumptive preovulatory follicle was removed via colpotomy with a chain ecraseur at 0 ($n = 5$),

12 ($n = 5$), 24 ($n = 5$), 30 ($n = 5$), 33 ($n = 5$), 36 ($n = 6$) and 39 h ($n = 5$) after hCG, as described by Vaughan (1988) and Sirois *et al.* (1989). Twenty-seven mares were used for a total of 36 unilateral ovariectomies. Animals used for two consecutive unilateral ovariectomies were allowed a recovery period of more than 40 days and showed at least one normal oestrous cycle before the second surgery. During the procedure, neuroleptanalgesia was induced with a combination of xylazine (Rompun; 0.65 mg kg⁻¹, i.v.), butorphanol (Torbugesic; 0.005 mg kg⁻¹, i.v.) and detomidine (Dormosedan; 0.02 mg kg⁻¹, i.v.) according to Sirois and Dore (1997). The recovered ovary was kept in ice-cold Eagles's minimal essential medium (MEM) supplemented with penicillin (50 U ml⁻¹)–streptomycin (50 µg ml⁻¹; Gibco), L-glutamine (2.0 mmol l⁻¹; Gibco) and non-essential amino acids (0.1 mmol l⁻¹; Gibco). The preovulatory follicle was dissected from the surrounding ovarian tissue with a scalpel, and pieces of the follicle wall were fixed in 10% (v/v) neutral buffered formalin. All animal procedures were approved by the institutional animal use and care committee. No complications were observed during the recovery period after surgery, with the exception of one mare that suffered from a severe peritonitis.

Histology

Formalin-fixed tissues were embedded in paraffin wax and 3 µm sections were prepared and stained with haematoxylin–eosin–safran (HES). Alcian blue (pH 2.5) staining was also performed on selected sections. The morphology of the follicle wall and its cellular constituents were characterized under light microscopy. The following parameters were quantified in the follicle wall: (i) the number of cells and thickness of the granulosa layer; (ii) the number of cells and thickness of the theca interna layer; (iii) the number of blood vessels (capillaries, venules and small veins) in the theca interna and theca externa; and (iv) the number of eosinophils in the theca interna and theca externa. Five representative high-power fields ($\times 400$) were selected on each section to determine these parameters. In addition, the following parameters were evaluated in a semi-quantitative way in the follicle wall: (i) degree of hyperaemia (0 = absent, 1 = little, 2 = moderate, 3 = marked); (ii) degree of oedema (0 = absent, 1 = light, 2 = moderate, 3 = marked); (iii) presence of haemorrhage (0 = absent, 1 = few, 2 = numerous); and (iv) presence of acid mucosubstances as detected with alcian blue staining (pH 2.5) (0 = absent, 1 = little, 2 = moderate, 3 = high, 4 = very high). Five representative fields ($\times 100$) were selected on each section to determine these parameters.

Statistical analyses

The effect of time after hCG treatment on the number of cells and thickness of the granulosa and theca layers, and on the number of blood vessels and eosinophils in the theca layers was tested by one-way analysis of variance (ANOVA). When ANOVAs indicated significant differences ($P < 0.05$),

Fig. 1. Histology of equine preovulatory follicles during hCG-induced ovulation. Sections of follicle walls were prepared from equine preovulatory follicles isolated during oestrus at (a) 0, (b) 12, (c) 24, (d) 30, (e) 36 and (f) 39 h after hCG treatment. Note the loosening of the granulosa cell layer (c–f), thinning of the theca interna (d–f), developing oedema (d–f) and progressive vascular changes (e,f). Scale bar represents 25 μ m.

multiple comparisons of means were made using the Dunnett's test to compare different time points after hCG with 0 h. The effect of time after hCG treatment on the degree of hyperaemia and oedema, and on the presence of haemorrhage and acid mucosubstances was tested by

Kruskal–Wallis one-way ANOVA by ranks, followed by Wilcoxon rank sum tests to compare different times after hCG with 0 h. Analyses were performed with JMP software (Statistical Analysis System Institutes, Cary, NC). Data are presented as mean $\pm$ SEM ($n = 5$ –6 follicles per time point).

Fig. 2. Changes in the granulosa cell layer of equine preovulatory follicles after hCG treatment. (a) Effect of hCG on the thickness of the granulosa cell layer. Sections of follicle walls of equine preovulatory follicles were isolated between 0 and 39 h after hCG, and the thickness of the granulosa cell layer was measured. (b) Time- and gonadotrophin-dependent accumulation of acid mucosubstances. The relative amount of acid mucosubstances in the granulosa cell layer was estimated using an arbitrary scale. Results are presented as mean $\pm$ SEM ($n = 5$ –6 follicles per time point). Bars with an asterisk are significantly different from time 0 h ($P < 0.01$).

Results

Macroscopic characteristics of equine preovulatory follicles

Thirty-six equine preovulatory follicles were isolated during oestrus between 0 and 39 h after hCG, including five follicles at 0, 12, 24, 30, 33 and 39 h, and six follicles at 36 h after hCG treatment. The overall mean follicle diameter was 39.4 ± 0.5 mm, and no significant difference was observed across time points after hCG ($P > 0.05$). All preovulatory follicles recovered between 0 and 36 h were intact, whereas two of the five follicles obtained 39 h after hCG ruptured accidentally during the procedure. Follicles isolated at 39 h after hCG appeared easily deformable at the ultrasound examination before the surgery and were very soft upon palpation, indicating imminent ovulation. After follicles were dissected and opened with scissors, a clear circular haemorrhagic area of approximately 1.0–1.5 cm in diameter located towards the ovulatory fossa was observed in the wall of four of the five follicles obtained 39 h after hCG. Only one follicle isolated at 36 h contained a similar haemorrhagic region, whereas none was observed in follicles at earlier time points. A mucoid substance coating the luminal side of the

follicle was observed after hCG treatment. This mucoid substance became more apparent macroscopically at 30 h after hCG, and made the granulosa cell layer sticky.

Microscopic gonadotrophin-induced changes in the granulosa cell layer

The administration of hCG induced marked microscopic changes in the granulosa cell layer (Fig. 1). Before hCG treatment (0 h), the granulosa cell layer was composed of four to nine compact layers of small elongated cells. These cells contained a small dark-staining nucleus that was often located in a basal position. Mitotic figures were occasionally observed (0–2 mitoses per field; $\times 400$). Gonadotrophin treatment caused a marked loosening of the granulosa cell layer that resulted in a significant increase in the thickness of the layer from 77.8 ± 4.8 to 158.8 ± 4.8 μm between 0 and 39 h after hCG ($P < 0.01$; Figs 1 and 2). The marked loosening of the granulosa cell layer was the result of accumulation of mucoid material between the cells. Alcian blue staining revealed that this material was composed of acid mucosubstances (Fig. 3a,b). The accumulation was progressive, but was greatest at 36 and 39 h after hCG ($P < 0.01$; Fig. 2b). No mitotic figures were observed in the granulosa cell layer after hCG treatment. The mean number of layers of granulosa cells increased between 0 and 24 h after hCG (5.5 ± 0.2 and 6.4 ± 0.2 cells, respectively; $P < 0.01$), but did not increase thereafter.

Microscopic gonadotrophin-induced changes in theca layers

The theca interna of equine preovulatory follicles isolated before hCG treatment (0 h) was relatively thick (85.5 ± 4.5 μm ; Fig. 4), and composed of plump polyhedral cells containing a light-staining nucleus (Fig. 1). The layer was well vascularized, containing numerous vessels (6.6 ± 0.5 vessels per field; $\times 400$; Fig. 5). The theca externa was less vascularized (3.5 ± 0.4 vessels per field; $\times 400$; Fig. 5) and composed of small fibroblasts. The boundary between theca interna and theca externa was easily detectable at this time point (0 h after hCG), because of marked differences in cell morphology.

The administration of hCG caused marked changes in the morphology of theca layers (Fig. 1). A significant thinning of the theca interna layer was observed after hCG treatment (Fig. 4). Fewer cell layers were present, theca interna cells appeared smaller than before hCG, and the presence of occasional pyknotic cells was noted at 36 and 39 h after hCG. In addition, there was marked infiltration of theca layers by eosinophils. Equine eosinophilic leucocytes were easily recognizable by the very large and tightly packed cytoplasmic granules that give the cells a raspberry-like appearance (Jain, 1986). No eosinophils were observed in theca interna and theca externa of preovulatory follicles isolated between 0 and 24 h after hCG, but a rapid and significant increase in numbers occurred thereafter (Figs 3c,d and 6). The number of eosinophils per field ($\times 400$) in the theca interna increased from 0.65 ± 0.06 to 14.0 ± 1.5 between

Fig. 3. Accumulation of acid mucosubstances in the granulosa cell layer (a,b) and the infiltration of eosinophils in theca layers (c,d) in equine follicles during hCG-induced ovulation. Sections of follicle walls were prepared from equine preovulatory follicles isolated at (a) 0, (c) 30 and (b,d) 39 h after hCG, and stained with haematoxylin–eosin–safran (a,b) or alcian blue, pH 2.5 (c,d). Arrowheads (d) indicate five or > 40 eosinophils that are present in this field. Scale bars represent 25 μ m.

30 and 39 h after hCG, respectively ($P < 0.05$). Numerous eosinophils were observed migrating along the endothelium of small vessels and into the extravascular compartment (Fig. 3c,d).

Marked vascular changes were observed in theca layers after hCG treatment (Fig. 1). These changes included significant increases in the degree of oedema, haemorrhages and hyperaemia, which were maximal at 36 and 39 h after hCG (Fig. 7). The number of blood vessels (per field; $\times 400$) in the theca interna and theca externa was significantly increased at 33, 36 and 39 h after hCG, respectively ($P < 0.01$; Fig. 5).

Discussion

This study is the first to provide an in-depth characterization of the sequential cellular and vascular changes that occur in equine follicles before ovulation. In contrast to detailed reports in other species, histological investigations of ovarian structures in mares have been limited and have focused primarily on the corpus luteum (Harrison, 1946; Van Niekerk *et al.*, 1975; Levine *et al.*, 1979). In this study, changes in the

microscopic anatomy of the equine follicle were characterized using 36 presumptive preovulatory follicles identified by ultrasonography and isolated during oestrus at specific times after the administration of an ovulatory dose of hCG. The induction of ovulation was associated with extensive remodelling of the granulosa cell layer. The marked expansion of the layer was, to an extent, reminiscent of a process thought to be limited to the cumulus–oocyte complex (Salustri *et al.*, 1996). Although a loosening of granulosa cells just before ovulation has been reported in other species (Priedkalns *et al.*, 1968; Parr, 1974; Cherney *et al.*, 1975), the degree of expansion observed in mares appears unique. The precise nature of the mucosubstances secreted during cellular expansion remains to be determined, but are likely to include members of the glycosaminoglycan family (hyaluronan, heparan sulfate or dermatan sulfate) as observed in other species (Grimek *et al.*, 1984; Bellin and Ax, 1987; Jackson *et al.*, 1991; Chen *et al.*, 1993; Boushehri *et al.*, 1996; Tirone *et al.*, 1997). The remodelling of the granulosa layer is not restricted to hCG treatment since it has also been observed in equine preovulatory follicles isolated during late oestrus (Ginther, 1992; J. Sirois, unpublished). A consequence

Fig. 4. Changes in the theca interna layer of equine preovulatory follicles after hCG treatment. Effects of hCG on (a) the number of cells and (b) the thickness of the theca interna layer were determined in sections of follicle walls of preovulatory follicles isolated between 0 and 39 h after hCG. Results are presented as mean $\pm$ SEM ($n = 5-6$ follicles per time point). Bars with an asterisk are significantly different from time 0 h ($P < 0.01$).

of the large amount of mucosubstances produced is the progressive entrapment of granulosa cells. This entrapment makes isolation of equine cells for *in vitro* studies particularly tedious during the late stages of ovulation, and repeated centrifugations and washes are required.

In a study that focused primarily on the histology of the developing equine corpus luteum, Van Niekerk *et al.* (1975) reported that theca interna cells degenerate at ovulation in mares and, therefore, in contrast to other species, do not contribute to the luteal tissue. The results of the present study are consistent with this finding, as a marked thinning of the theca interna and occasional cell pyknosis were observed after hCG treatment. These changes were observed primarily at the end of ovulation, and probably relate to the requirement for adequate thecal androgen production during the follicular phase to maintain follicular oestradiol biosynthesis. A potential biochemical consequence of thecal degeneration was indicated by the marked decrease in transcripts encoding the steroidogenic acute regulatory protein (StAR) in these cells at 36 and 39 h after hCG (Kerban *et al.*, 1999). However, the putative degeneration of the theca interna in mares remains obscure, since it has not been reported in other species. Future investigations should concentrate on determining the nature of the process

Fig. 5. Number of thecal blood vessels in equine follicles before ovulation. Sections of follicle walls of equine preovulatory follicles were isolated between 0 and 39 h after hCG, and the number of blood vessels per field ($\times 400$; $n = 5$ fields per follicle) was determined in (■) theca interna and (□) theca externa. Results are presented as mean $\pm$ SEM ($n = 5-6$ follicles per time point). Bars with an asterisk are significantly different from time 0 h ($P < 0.01$).

Fig. 6. Time course of eosinophil infiltration into theca interna and theca externa of equine follicles after hCG treatment. Sections of follicle walls of equine preovulatory follicles were isolated between 0 and 39 h after hCG, and the number of eosinophils per field ($\times 400$) was determined in (■) theca interna and (□) theca externa. Results are presented as mean $\pm$ SEM ($n = 5-6$ follicles per time point). Bars with an asterisk are significantly different from time 0 h ($P < 0.01$).

involved (apoptosis versus necrosis), as well as the cellular and molecular mechanisms responsible for cell death.

This is the first study to report the extensive migration of eosinophilic leucocytes into the equine follicle wall during ovulation. The attraction of white blood cells from the circulation into theca layers of preovulatory follicles has been reported in other species (Cavender and Murdoch, 1988; Standaert *et al.*, 1991; Gerdes *et al.*, 1992; Brannstrom *et al.*, 1993), supporting their role in the inflammatory-like

Fig. 7. Degree of (a) haemorrhage, (b) oedema and (c) hyperaemia in equine follicles after hCG treatment. Sections of follicle walls of equine preovulatory follicles were isolated between 0 and 39 h after hCG, and the degree of haemorrhage, oedema and hyperaemia were determined using an arbitrary scale. Results are presented as mean of 5–6 follicles per time point. Bars with an asterisk are significantly different from time 0 h ($P < 0.01$).

response in the ovary at ovulation. There appear to be species-related differences in the leucocyte subtype attracted into the preovulatory follicle; eosinophils are predominant in equine (this study) and pig follicles (Standaert *et al.*, 1991) and neutrophils are the principal leucocyte detected in rat (Brannstrom *et al.*, 1993) and rabbit follicles (Gerdes *et al.*, 1992). Eosinophils were first detected at 33 h after hCG in equine follicles, which coincides precisely with the gonadotrophin-dependent induction of prostaglandin synthesis in granulosa cells before ovulation (Watson and Sertich, 1991; Sirois and Dore, 1997). However, a causal relationship between prostaglandin synthesis and leucocyte migration is not supported by studies in other species (Espey *et al.*, 1981; Chun *et al.*, 1993).

Leucocyte chemoattractant activities have been detected in fluid and cellular extracts of bovine (Sirotkin and Luck, 1995), equine (Watson *et al.*, 1991), human (Seow *et al.*, 1988) and ovine follicles (Murdoch and McCormick, 1989). The finding of Watson *et al.* (1991) that there was no significant difference in the neutrophil chemotactic activity of fluid aspirated from equine preovulatory follicles between 0 and

36 h after hCG is consistent with results of the present study, since predominantly eosinophils, and not neutrophils, were attracted in the equine follicle. Although the precise nature of the follicular chemotactic signal has not been identified, studies in sheep (Murdoch and McCormick, 1989, 1993) and cattle (Sirotkin and Luck, 1995) indicate that potential candidates include collagen-like peptides, collagenase, ascorbic acid and calcium ions. However, the precise functions of the massive follicular eosinophilia in mares during ovulation remain uncertain. Depletion of circulatory eosinophils in sheep using prednisolone prevented the characteristic eosinophilia in preovulatory follicles, but did not affect ovulation (Murdoch and Steadman, 1991). Similarly, severe depletion of circulatory leucocytes in rats did not prevent ovulation (Chun *et al.*, 1993).

Marked vascular changes were observed in the equine follicle as ovulation approached, including an apparent increase in blood supply, hyperaemia, vasodilation, oedema and haemorrhages. These changes have been described in other species and helped to establish a clear parallel between the ovulation and a classic inflammatory reaction (Espey, 1980). However, attempts to identify the follicular mediators responsible for the gonadotrophin-induced vascular response have not produced consistent results (Goetz *et al.*, 1991; Tsafiriri *et al.*, 1993; Espey and Lipner, 1994). Prostaglandins are thought to play a key role during ovulation (Murdoch *et al.*, 1993; Priddy and Killick, 1993). The observation that female mice deficient for prostaglandin G/H synthase 2 (PGHS-2), the first rate-limiting enzyme in the biosynthetic pathway of prostaglandins from arachidonic acid (Herschman, 1996), have impaired ovulation (Dinchuk *et al.*, 1995; Lim H *et al.*, 1997) supports this proposal. In equine preovulatory follicles, a selective induction of PGHS-2 in granulosa cells beginning 30 h after hCG treatment is responsible for the marked increase in follicular prostaglandin synthesis before ovulation (Watson and Sertich, 1991; Sirois and Dore, 1997; Boerboom and Sirois, 1998). Increased concentrations of prostaglandins are likely to be involved in the vascular response observed in the present study, as well as in the proteolytic and collagenolytic activities needed for follicular rupture (Tsafiriri *et al.*, 1993).

In summary, this study is the first to characterize the cellular and vascular changes that occur in equine follicles during ovulation. Owing to the variable duration of the follicular phase in mares and the absence of a discrete LH preovulatory surge (Whitmore *et al.*, 1973; Stabenfeldt *et al.*, 1975; Alexander and Irvine 1982), exogenous gonadotrophin (hCG) was used to induce ovulation and enable the chronology of the follicular response to be determined. The main findings regarding ovulation in mares included a marked expansion of the granulosa cell layer, a putative degeneration of theca interna cells, and a pronounced follicular eosinophilia. Future studies are required to determine the cellular and molecular basis for these gonadotrophin-induced changes in equine follicles before ovulation.

This study was supported by Natural Sciences and Engineering Research Council of Canada Grant OGP0171135. A. Kerban was supported by a fellowship from Al-Fateh University.

References

- Alexander S and Irvine CHG (1982) Radioimmunoassay and *in vitro* bioassay of serum LH throughout the equine oestrous cycle *Journal of Reproduction and Fertility Supplement* 32 253–260
- Almadhidi J, Seralini GE, Fresnel J, Silberzahn P and Gaillard JC (1995) Immunohistochemical localization of cytochrome P450 aromatase in equine gonads *Journal of Histochemistry and Cytochemistry* 43 571–577
- Bellin ME and Ax RL (1987) Chemical characteristics of follicular glycosaminoglycans *Advances in Experimental Medicine and Biology* 219 731–735
- Bjersing L and Cajander S (1974) Ovulation and the mechanism of follicle rupture. I. Light microscopic changes in rabbit ovarian follicles prior to induced ovulation *Cell Tissue Research* 149 287–300
- Boerboom D and Sirois J (1998) Molecular characterization of equine prostaglandin G/H synthase-2 and regulation of its messenger ribonucleic acid in preovulatory follicles *Endocrinology* 139 1662–1670
- Boushehri I, Yadav MC and Meur SK (1996) Characteristics of proteoglycans of buffalo ovarian follicular fluid during maturation of follicles *Indian Journal of Biochemistry and Biophysics* 33 213–217
- Brannstrom M, Mayrhofer G and Robertson SA (1993) Localization of leukocyte subsets in the rat ovary during the periovulatory period *Biology of Reproduction* 48 277–286
- Byсков AG (1969) Ultrastructural studies on the preovulatory follicle in the mouse ovary *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie* 100 285–299
- Cavender JL and Murdoch WJ (1988) Morphological studies of the micro-circulatory system of periovulatory ovine follicles *Biology of Reproduction* 39 989–997
- Chen L, Russell PT and Larsen WJ (1993) Functional significance of cumulus expansion in the mouse: roles for the preovulatory synthesis of hyaluronic acid within the cumulus mass *Molecular Reproduction and Development* 34 87–93
- Cherney DD, Didio L and Motta P (1975) The development of rabbit ovarian follicles following copulation *Fertility and Sterility* 26 257–270
- Chun SY, Daphna-Iken D, Calman D and Tsafirri A (1993) Severe leukocyte depletion does not affect follicular rupture in the rat *Biology of Reproduction* 48 905–909
- Daels PF and Hughes JP (1993) The normal estrous cycle. In *Equine Reproduction* pp 121–132 Eds AO McKinnon and JL Voss. Lea & Febiger, Philadelphia
- Dinchuk JE, Car BD, Focht KJ *et al.* (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II *Nature* 378 406–409
- Driancourt MA and Palmer E (1984) Time of ovarian follicular recruitment in cyclic pony mares *Theriogenology* 21 591–600
- Duchamp G, Bour B, Combarous Y and Palmer E (1987) Alternative solutions to hCG induction of ovulation in the mare *Journal of Reproduction and Fertility Supplement* 35 221–228
- Espey LL (1967) Ultrastructure of the apex of the rabbit Graafian follicle during the ovulatory process *Endocrinology* 81 267–276
- Espey LL (1980) Ovulation as an inflammatory reaction – a hypothesis *Biology of Reproduction* 22 73–106
- Espey LL, Coons PJ, Marsh JM and LeMaire WJ (1981) Effect of indomethacin on preovulatory changes in the ultrastructure of the rabbit Graafian follicle *Endocrinology* 108 1040–1048
- Espey LL and Lipner H (1994) Ovulation. In *The Physiology of Reproduction* pp 725–780 Eds E Knobil and JD Neill. Raven Press, New York
- Gerdes U, Gravels M, Bergh A and Cajander S (1992) Localized increases in ovarian vascular permeability and leukocyte accumulation after ovulation in rabbits *Journal of Reproduction and Fertility* 95 539–550
- Ginther OJ (1992) Characteristics of the ovulatory season. In *Reproductive Biology of the Mare* pp 173–235. Equiservices, Wisconsin
- Goetz FW, Berndtson AK and Ranjan M (1991) Ovulation: mediators at the ovarian level. In *Vertebrate Endocrinology: Fundamentals and Biomedical Implications* pp 127–203 Eds PKT Pang, MP Schreibman and R Jones. Academic Press, New York
- Grimek HJ, Bellin ME and Ax RL (1984) Characteristics of proteoglycans isolated from small and large bovine ovarian follicles *Biology of Reproduction* 30 397–409
- Harrison RJ (1946) The early development of the corpus luteum in the mare *Journal of Anatomy* 8 160–166
- Herschman HR (1996) Review: prostaglandin synthase-2 *Biochimica et Biophysica Acta* 1299 125–140
- Jackson JA, Friberg AC and Bahr JM (1991) Preovulatory changes in glycosaminoglycans and collagen content in the stigma region of the follicle of the domestic hen *Biology of Reproduction* 45 301–307
- Jain NC (1986) The horse: normal hematology with comments on response to disease. In *Schalm's Veterinary Hematology* pp 140–177. Lea & Febiger, Philadelphia
- Kerban A, Boerboom D and Sirois J (1999) Human chorionic gonadotropin induces an inverse regulation of steroidogenic acute regulatory protein messenger ribonucleic acid in theca interna and granulosa cells of equine preovulatory follicles *Endocrinology* 140 667–674
- Levine H, Wight T and Squires E (1979) Ultrastructure of the corpus luteum of the cycling mare *Biology of Reproduction* 20 492–504
- Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM and Dey SK (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice *Cell* 91 197–208
- Martin GG and Talbot P (1987) Formation of the rupture site in preovulatory hamster follicles: morphological and morphometric analysis of thinning of the granulosa and thecal layers *Gamete Research* 17 303–320
- Murdoch WJ and Cavender JL (1987) Mechanisms of ovulation *Advances in Contraceptive Delivery Systems* 3 353–366
- Murdoch WJ and McCormick RJ (1989) Production of low molecular weight chemoattractants for leukocytes by periovulatory ovine follicles *Biology of Reproduction* 41 86–90
- Murdoch WJ, Hansen TR and McPherson LA (1993) A review – role of eicosanoids in vertebrate ovulation *Prostaglandins* 46 85–115
- Murdoch WJ and McCormick RJ (1993) Mechanisms and physiological implications of leukocyte chemoattraction into periovulatory ovine follicles *Journal of Reproduction and Fertility* 97 375–380
- Murdoch WJ and Steadman LE (1991) Investigations concerning the relationship of ovarian eosinophilia to ovulation and luteal function in the sheep *American Journal of Reproductive Immunology* 25 81–87
- Okamura H, Takenaka A, Yajima Y and Nishimura T (1980) Ovulatory changes in the wall at the apex of the human Graafian follicle *Journal of Reproduction and Fertility* 58 153–155
- Palmer E (1978) Control of the oestrous cycle of the mare *Journal of Reproduction and Fertility* 54 495–505
- Parr EL (1974) Histological examination of the rat ovarian follicle wall prior to ovulation *Biology of Reproduction* 11 483–503
- Pierson RA and Ginther OJ (1985) Ultrasonic evaluation of the preovulatory follicle in the mare *Theriogenology* 24 359–368
- Pierson RA and Ginther OJ (1987) Follicular population dynamics during the estrous cycle of the mare *Animal Reproduction Science* 14 219–231
- Priddy AR and Killick SR (1993) Eicosanoids and ovulation *Prostaglandins Leukotrienes and Essential Fatty Acids* 49 827–831
- Priedkalns J, Weber AF and Zemjanis R (1968) Qualitative and quantitative morphological studies of the cells of the membrane granulosa, theca interna and corpus luteum of the bovine ovary *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie* 85 501–520
- Salustri A, Camaioni A and D'Alessandris C (1996) Paracrine and endocrine regulation of cumulus expansion *Zygote* 4 313–315
- Seow WK, Thong YH, Waters MJ, Walters M and Cummins JM (1988) Isolation of a chemotactic protein for neutrophils from human ovarian follicular fluid *International Archives of Allergy and Applied Immunology* 86 331–336
- Sirois J, Ball BA and Fortune JE (1989) Patterns of growth and regression of ovarian follicles during the oestrous cycle and after hemiovariectomy in mares *Equine Veterinary Journal Supplement* 8 43–48
- Sirois J, Kimmich TL and Fortune JE (1991) Steroidogenesis by equine preovulatory follicles: relative roles of theca interna and granulosa cells *Endocrinology* 128 1159–1166
- Sirois J and Dore M (1997) The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process *Endocrinology* 138 4427–4434
- Sirotkin AV and Luck MR (1995) Potential leukocyte attractants in the bovine peri-ovulatory ovary *Reproduction Nutrition Development* 35 675–683
- Stabenfeldt GH, Hughes JP, Evans JW and Geschwind II (1975) Unique aspects of the reproductive cycle of the mare *Journal of Reproduction and Fertility Supplement* 23 155–160
- Standaert FE, Zamora CS and Chew BP (1991) Quantitative and qualitative changes in blood leukocytes in the porcine ovary *American Journal of Reproductive Immunology* 25 163–168
- Thibault C and Levasseur MC (1988) Ovulation *Human Reproduction* 3 513–523

- Townson DH and Ginther OJ** (1987) Duration and pattern of follicular evacuation during ovulation in the mare *Animal Reproduction Science* **15** 131–138
- Townson DH and Ginther OJ** (1989) Ultrasonic characterization of follicular evacuation during ovulation and fate of the discharged follicular fluid *Animal Reproduction Science* **20** 131–141
- Tirone E, D'Alessandris C, Hascall VC, Siracusa C and Salustri A** (1997) Hyaluronan synthesis by mouse cumulus cells is regulated by interactions between follicle-stimulating hormone (or epidermal growth factor) and a soluble oocyte factor (or transforming growth factor beta 1) *Journal of Biological Chemistry* **272** 4787–4794
- Tsafiri A, Chun SY and Reich R** (1993) Follicular rupture and ovulation. In *The Ovary* pp 227–244 Eds EY Adashi and PCK Leung. Raven Press, New York
- Van Niekerk CH, Morgenthal JC and Gerneke WH** (1975) Relationship between the morphology of, and progesterone production by, the corpus luteum of the mare *Journal of Reproduction and Fertility Supplement* **23** 171–175
- Vaughan JT** (1988) The female genital system. In *Textbook of Large Animal Surgery* pp 581–583 Ed. FW Oehme. Williams and Wilkins, Baltimore
- Watson ED and Sertich PL** (1991) Concentrations of arachidonic metabolites, steroids and histamine in preovulatory horse follicles after administration of human chorionic gonadotropin and the effect of intrafollicular injection of indomethacin *Journal of Endocrinology* **129** 131–139
- Watson ED, Sertich PL and Zanecosky HG** (1991) Detection of chemotactic factors in preovulatory follicular fluid from mares *American Journal of Veterinary Research* **52** 1412–1415
- Whitmore HL, Wentworth BC and Ginther OJ** (1973) Circulating concentrations of luteinizing hormone during estrous cycle of mares as determined by radioimmunoassay *American Journal of Veterinary Research* **34** 631–636