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Review article

Passage of postovulatory follicular fluid into the peritoneal cavity and the effect on concentrations of circulating hormones in mares

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Abstract

Reported data were reviewed and reexamined to evaluate the concept that most of the follicular fluid enters the peritoneal cavity at ovulation in mares and transiently alters the circulating concentrations of LH, FSH, estradiol, and inhibin. A transrectal ultrasonographic study supported the hypothesis that the large volume (40–50 ml) of evacuated follicular fluid passes through the infundibular fimbriae into the peritoneal cavity. A spike in circulating inhibin and a decrease in the rate of reduction in circulatory estradiol occurs at ovulation. Simultaneously, a disruption occurs in the increasing concentrations of the ovulatory LH surge and in the FSH surge that begins before ovulation. The concept was further supported by the present finding that the estradiol content of follicular fluid within a few hours before ovulation is equivalent to the amount reported to be needed for a negative effect on LH and for a synergistic negative effect of estradiol and inhibin on FSH.

Keywords: Follicular fluid; Gonadotropins; Mares; Ovarian hormones; Peritoneum

1. Introduction

Follicular fluid of the preovulatory follicle contains a myriad of biologically active factors and hormones, including steroid hormones (estrogens, progestagens, androgens), peptide hor-

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mones (inhibin, activins, follistatin), prostaglandins, cytokines, insulin-like growth factors and their binding proteins, and many other factors (Baka and Malamitsi-Puchner, 2006; Webb and Campbell, 2007). The substances found in follicular fluid determine the fate of the follicle and oocyte by interacting with systemic factors (Webb and Campbell, 2007). The preovulatory follicle has been studied extensively in many species, owing to a single cell, the oocyte, and its fundamental role in perpetuation of the species. However, comparatively scant attention has been paid to the postovulatory fate and function of the substantial volume of follicular fluid and its diverse content of active factors. This review considers the concept that the postovulatory oocyte and follicular fluid follow different routes in mares. The oocyte enters the oviduct, whereas most of the follicular fluid enters the peritoneal cavity and alters the circulating concentrations of LH, FSH, inhibin, and estradiol.

2. Follicle evacuation and infundibular fluid

A portion of the funnel-shaped ovarian end or infundibulum of the oviduct is attached to the cranial aspect of the ovulation fossa (Ginther, 1992). The infundibular processes or fimbriae of the remaining portion are juxtaposed to the remainder of the fossa during ovulation. A preovulatory collection of fluid external to the ovary in the infundibular area has been detected by transrectal ultrasonography (Townson and Ginther, 1989). In a detailed study, a fluid-filled infundibulum was detected by ultrasonic imaging in 46% of oviducts on Day −10, 88% on Day −3, and 8% on Day 7 (Gastal et al., 2007). The fluid pocket ranged from an equivalent of 5–20 mm in diameter (Fig. 1). Floating infundibular folds or fimbriae were detected by ballottement within the accumulation of fluid. The source of the fluid has not been determined but presumably originates from the oviduct, peritoneum, or both. As ovulation approaches, a bulge at the apex of the follicle can be detected at the ovulation fossa by laparoscopy (Witherspoon and Talbot, 1970) or by ultrasonic imaging (Carnevale et al., 1988; Gastal et al., 2006). This thin-walled and relatively avascular portion of the preovulatory follicle (Ginther et al., 2007a) separates the infundibular fluid pocket and the follicular antrum.

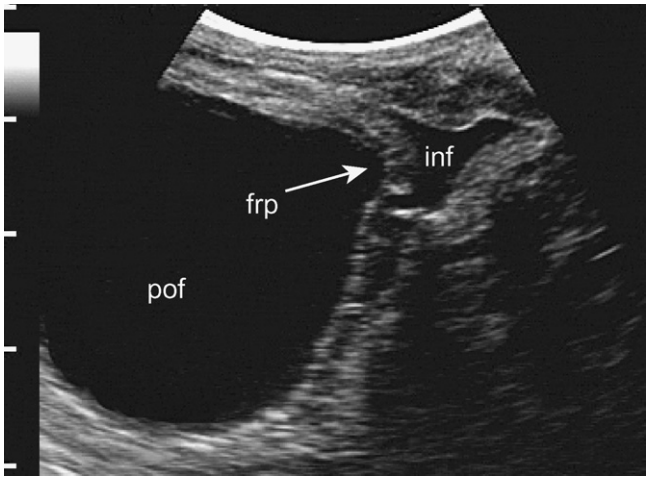

Fig. 1. Ultrasonogram from transrectal imaging, illustrating the preovulatory follicle (pof), apex or future rupture point (frp), and accumulation of extra-ovarian fluid in the infundibular (inf) area. The distance between graduation marks (left margin) is 10 mm.

The follicular fluid enters the infundibular fluid accumulation during follicle evacuation at ovulation. The first chance observations of continuous follicle evacuation by transrectal ultrasonography were made in mares (Ginther and Pierson, 1984). In subsequent planned studies, two distinctive evacuation patterns were observed during continuous monitoring by ultrasonography (Townson and Ginther, 1987, 1989). Abrupt and gradual evacuation involved a 90% and 50% decrease, respectively, in the antral area of the follicle within 60 s. Gradual evacuation required an additional 6–7 min. Similar distinct abrupt versus gradual evacuation patterns have been reported for cattle (Kot and Ginther, 1999).

3. Entry of follicular fluid into the abdomen

Recent studies have indicated that most of the follicular fluid passes through the fimbriae into the peritoneal cavity, leaving the oocyte and its granulosa investment for entry into the oviduct. The majority of the follicular fluid passes into the abdomen, based on the specific testing of this hypothesis by continuous ultrasonic imaging in mares (Townson and Ginther, 1989). During follicle evacuation, fluid in the oviduct was not detectable, except for the fluid accumulation in the infundibular area that was present before follicle evacuation. The fluid pocket in the infundibulum did not enlarge and the remaining oviduct did not become detectable from fluid distention during follicle evacuation. It seems unlikely that the large volume of follicular fluid could pass through the restricted lumen of the isthmus into the uterus during follicle evacuation, especially without dilation of the ampulla. The hypothesis that most of the fluid passed or was immediately filtered between the surface of the ovulation fossa and the oviductal fimbriae into the peritoneal cavity was thereby supported.

The proportion of follicular fluid that enters the oviduct beyond the infundibulum is unknown for any species, but was small enough to be undetected by ultrasonic imaging during and after follicular evacuation in mares, despite the large volume of follicular fluid (Townson and Ginther, 1989). The limited available information on the passage of follicular fluid into the oviduct and peritoneal cavity in other species has been discussed (Hunter, 2003). Most notably, it has been concluded that less than 1% of the follicular fluid in pigs was retained in the oviduct shortly after ovulation, based on the progesterone content of the discharged follicular fluid (Hansen et al., 1991). The role of the postovulatory follicular fluid in continued oocyte development, transport, and fertilization is not obligatory, as indicated by fertilization of equine oocytes surgically transferred from follicle to ampulla (Carnevale and Ginther, 1995) and by ovulation and fertilization after all follicular fluid has been removed by aspiration (Palmer et al., 1997).

4. Peritoneal absorption of follicular-fluid inhibin

The first speculative proposal that hormones in the discharged follicular fluid may be absorbed from the abdomen into the systemic circulation was made in association with the finding that a distinct spike in circulating immunoreactive inhibin occurred on the day of ovulation in mares (Bergfelt et al., 1991). The greater circulating concentration of inhibin at the time of ovulation has been confirmed (Roser et al., 1994; Nagaoka et al., 1999). Subsequent studies used ovulation detection and blood sampling every 4 h (Nambo et al., 2002b) or ovulation detection at 32 and 48 h after hCG treatment and blood sampling every hour (Nambo et al., 2002a, 2006). Inhibins increased immediately after ovulation and returned to basal concentrations in 12 h, but an increase in estradiol and a decrease in FSH were not detected. Sampling of peritoneal fluid showed inhibin

A concentrations that were 300 times greater immediately after ovulation than at other stages of estrus (Nambo et al., 2006). Another approach involved injection of follicular fluid collected from slaughterhouse ovaries into the peritoneal cavity (Nambo et al., 2002b) or infusing fluid from the preovulatory follicle onto the peritoneal surface of the ovary (Nambo et al., 2006). Both approaches increased the circulating concentrations of inhibin, and the preovulatory follicular fluid also decreased the FSH concentrations. In this regard, it has been long known that intravenous treatment with whole or steroid-free follicular fluid suppresses the circulating concentrations of FSH in mares that were ovariectomized, having estrous cycles, and pregnant (Miller et al., 1979, 1981; Bergfelt and Ginther, 1985, 1986).

5. Follicular-fluid estradiol and circulating LH and FSH

In a study using sampling every 24 h with a large number of periovulatory periods ($n = 66$), a transient reduction in the rate of increase in FSH and LH seemed to occur between Days −1 and 0 (Jacob et al., 2007) but was not statistically evaluated. A similar transient change or disruption in the FSH and LH surges was not apparent in studies done at 24-h intervals, using fewer periovulatory periods (Ginther et al., 2005, 2006). A recent study of preovulatory development of follicles involved collection of blood samples and ultrasonic scanning every 12 h in 18 single-ovulating mares (Ginther et al., 2008). Results for Days −1 to 2 (ovulation = Day 0) are germane to this report and are depicted (Fig. 2). The gradual preovulatory mean increase in each gonadotropin was temporarily disrupted at ovulation. Concentration of LH and FSH increased significantly between Days −1 to −0.5 and Days 0.5 to 1 but not between Days −0.5 and 0.5. Concentration of estradiol decreased significantly between Days −1 and −0.5 and between Days 0 and 0.5, but the decrease between Days −0.5 and 0 was not significant. Ovulation was indicated by a collapsed follicle, and therefore actual discharge of fluid occurred sometime between Day −0.5 and Day 0. Clearly, examinations every 12 h were far more effective than every 24 h in detecting disruptions in hormone concentrations in association with ovulation.

The transient disruption in the LH increase can be attributed to discharge of the estradiol content of the follicular fluid into the peritoneal cavity followed by absorption into the circulatory system. A negative effect of estradiol on LH is consistent with the reported temporal relationships of a slower increase in LH in the ovulating surge during the increase in estradiol, followed by a more rapid LH increase after the peak of the estradiol surge (Ginther et al., 2006). A negative effect of the follicles (Ginther et al., 2005) and specifically estradiol (Ginther et al., 2007b) on LH throughout the ovulatory LH surge has been demonstrated by follicle ablations or treatment with exogenous estradiol. Finding an increase in circulating estradiol at the time of ovulation likely was thwarted by an incompatibility between the length of time involved for absorption from the abdomen (relatively rapid) compared with the length of the intervals between collections of blood samples (relatively long). The peritoneum is a serous membrane that lines the abdominal cavity and covers the abdominal viscera, thereby providing a vast surface area for absorption (König and Liebich, 2007). After follicular-fluid estradiol enters the peritoneal cavity, the duration of the resulting increase in circulating concentrations may be shorter than the 4 h reported after a single intramuscular injection of a physiologic dose of estradiol in an oil vehicle (Ginther et al., 2007b). In contrast, LH concentrations remained depressed for >8 h after an injection of estradiol (Miller et al., 1981). These considerations may account for the detection of a disruption in LH concentrations in periovulatory studies, despite the absence of a detected estradiol increase. Progesterone, which also has a negative effect on LH (Gastal et al., 1999a), increases slightly by the day of ovulation (Jacob et al., 2007), but whether the progesterone on Day 0 originates from peritoneal absorption

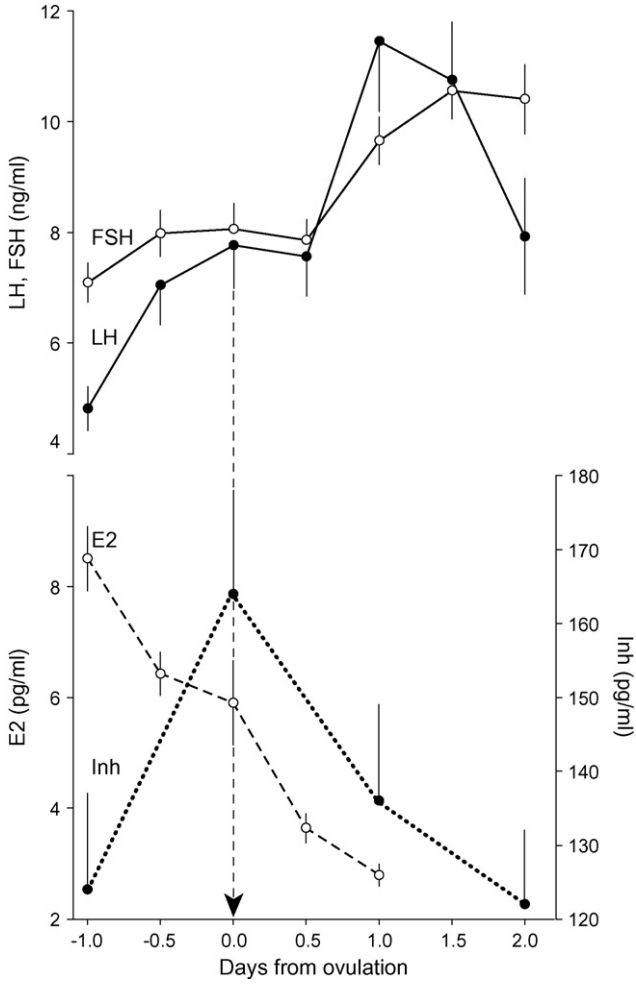

Fig. 2. Mean ± S.E.M. for periovulatory concentrations of LH, FSH, estradiol (E2), and immunoreactive inhibin (Inh). The increases in concentrations of LH and FSH are disrupted in temporal association with ovulation. Significant increases in LH and FSH occurred between Days −1 and −0.5 and between Days 0.5 and 1, but not between Days −0.5 and 0.5. A significant decrease in E2 occurred between adjacent half days, except between Days −0.5 and 0. Inhibin reached a peak on Day 0. These data are consistent with the discharge of follicular fluid with its E2 and inhibin content into the peritoneal cavity, resulting in a disruption in the gonadotropin surges. Data for LH, FSH, and E2 (Ginther et al., 2008) and for inhibin (Bergfelt et al., 1991) were adapted from published reports.

from the follicular fluid or from the beginning of production by the developing luteal cells is not known.

The well documented spike in ir-inhibin concentrations in association with evacuation of the follicle at ovulation has been attributed to the discharge of follicular fluid into the abdomen with absorption of the inhibin component into the circulation, as described in Section 4. This would at least partly account for the depicted transient suspension in the FSH increase between Days −0.5 and 0.5 (Fig. 2). In addition, a negative effect of estradiol on FSH is consistent with the beginning of a preovulatory increase in FSH concentrations concomitantly with a decrease in

estradiol concentrations (Jacob et al., 2007). A negative effect of estradiol on FSH has been demonstrated by follicle ablations (Gastal et al., 1999b) or treatment with estradiol (Donadeu and Ginther, 2003). In ovariectomized mares, charcoal-extracted follicular fluid (steroid free) caused a decrease in FSH, and estradiol-17β caused a decrease in both FSH and LH (Miller et al., 1979). The decrease in FSH was enhanced when the two treatments were given simultaneously. Thus, the synergistic effect of estradiol and inhibin from the follicular fluid that entered the peritoneal cavity more completely accounts for the transient suppression of the incline in FSH. Furthermore, the rebound in FSH after the transient disruption is consistent with a rebound in FSH 1 or 2 days after administration of a proteinaceous fraction of follicular fluid in mares (Bergfelt and Ginther, 1985).

6. Estradiol content of follicular fluid

The indications that estradiol in the discharged follicular fluid causes the transient fluctuations in the LH surge are indirect and temporal. Further support depends on a demonstration that the quantity of estradiol in the discharged follicular fluid is adequate for LH suppression. Therefore, the concentration of estradiol in follicular fluid was studied in nine mares at impending ovulation (unpublished). Ovulation was expected to occur in a few hours or less, based on the development of serrated granulosa (Gastal et al., 2006). The concentration was 1769 ± 73 ng/ml. The diameter of the preovulatory follicle on Day −1 in the experiment (Ginther et al., 2008) cited in Section 5 was 44.0 mm (equivalent to a volume of 44.6 ml). The calculated total estradiol content was 81 μg (1.8 μg/ml × 45 ml). A single intramuscular injection of 50 μg of estradiol resulted in a detectable increase in the blood, and a dose of 100 μg (smallest dose used) decreased the circulating concentrations of LH on various days throughout the ovulatory LH surge, including on the day of ovulation (Ginther et al., 2007b). These calculations involve the same pony herd and assay system used in the experiment (Ginther et al., 2008) cited in Section 5. The results indicate that the estradiol content of the discharged follicular fluid on the day of ovulation is adequate for disrupting the LH surge and contributing to disruption of the FSH increase, if absorbed rapidly from the peritoneal cavity (Section 5).

7. Conclusions

Reexamination and review of published data indicate that most of the follicular fluid discharged at ovulation in mares passes through the infundibular fimbriae into the peritoneal cavity. Estradiol and inhibin are then absorbed into the systemic circulation and transiently disrupt the increases in LH and FSH associated with the periovulatory surges. Assay of the estradiol concentrations of follicular fluid during the few hours before expected ovulation indicated that the estradiol content was equivalent to the amount required for a negative effect on LH at the time of ovulation.

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