Relationship Between Follicle Size and Concentrations of Steroids and Insulin-like Growth Factor (IGF)-I in Mares

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Story in Brief

The objective of this study was to determine if stage of the estrous cycle or follicle diameter influences concentrations of steroids or IGF-I in follicular fluid of mares. Follicular fluid and granulosa cells from small (<15 mm), medium (16-25 mm) and large (>25 mm) follicles were collected from luteal (n=6) and follicular (n=8) phase mares. Follicular fluid estradiol concentrations differed with estrous cycle stage and follicle diameter. In contrast, progesterone levels were not affected by either estrous cycle stage or follicle diameter. Concentrations of IGF-I did not differ with estrous cycle stage, but increased with follicle diameter. These results indicate that estrous cycle stage and(or) follicle diameter influence concentrations of steroids and IGF-I in follicular fluid of mares.

Key Words: Follicular Growth, Mare, Estradiol, Follicular Fluid, Insulin-like Growth Factor

Introduction

In the mare, one follicle is selected from a cohort of follicles to become dominant. After selection, the dominant follicle continues to grow until ovulation, while the remaining cohort, or subordinate follicles become atretic and regress (for review see Ginther, 2000). Limited information is available regarding the physiological mechanism of follicle selection and maturation in the mare. During preovulatory follicular development in the estrous mare, follicular fluid IGF-I levels increase (Spicer et al., 1991), but changes in follicular fluid IGF-I over a wider range of follicle sizes in the mare have not been reported. Therefore, the specific objectives of the present experiment were to determine whether stage of the estrous cycle or follicle diameter influences concentrations of steroids and IGF-I in follicular fluid of mares.

Materials and Methods

In late May a total of 28 ovaries were obtained at a commercial abattoir from 14 mares of various breeds, ages and sexual maturity. The mares were classified as either in the luteal (n = 6) or follicular (n = 8) phase based on gross ovarian morphology; ovaries with a viable (vasculature visible) corpus luteum (CL) were classified as being in the luteal phase, and ovaries with large follicles and a corpus albicans or regressing CL (as indicated by pale color and little or no vascularity with a diameter < 20 mm) were classified as being in the follicular phase. Follicular fluid from individual follicles were collected separately using needles and syringes. After granulosa cells were separated from follicular fluid by centrifugation (220 x g for 5 to 7 min), individual follicular fluid samples were frozen at –80 °C.

Concentrations of progesterone (P_4) and estradiol (E_2) in follicular fluid were determined with a double-antibody radioimmunoassays (RIA) as previously described (Stewart et al., 1996; Bridges et al., 2002). Concentrations of androstenedione (A_4) in follicular fluid were determined using

solid-phase RIA kits (ICN Biomedicals, Costa Mesa, CA) as previously described (Stewart et al., 1996; Bridges et al., 2002). Concentrations of IGF-I in follicular fluid were determined with a double-antibody RIA after acid-ethanol extraction (16 h at 4°C) as previously described (Spicer et al., 1991).

Data were analyzed using PROC MIXED of SAS. Linear regressions were performed for each response variable (E_2, P_4, A_4, and IGF-I) using the variable "SIZE" as the independent variable. Separate regressions were performed for each stage (Luteal or Follicular). Dummy variables for each response were used to assess whether the slopes associated with each stage were significantly different. If the slopes associated with the two stages were not statistically significant, then a model to assess the difference in the intercepts for the two stages was performed.

Results

Follicular fluid estradiol concentration differed during the follicular phase as compared to the luteal phase ($P<0.05$). Fitted lines for both the follicular and luteal phases are in Figure 1. During the follicular phase, estradiol concentration increased with an increase in follicle diameter ($P<0.05$). In contrast, follicular diameter was not related to estradiol concentration during the luteal phase ($P>0.10$). In contrast to estradiol, progesterone concentration in follicular fluid averaged 28.9 ± 3.8 ng/mL and did not differ ($P>0.10$) between the follicular and luteal phase, nor was it affected ($P>0.10$) by follicle diameter (data not shown).

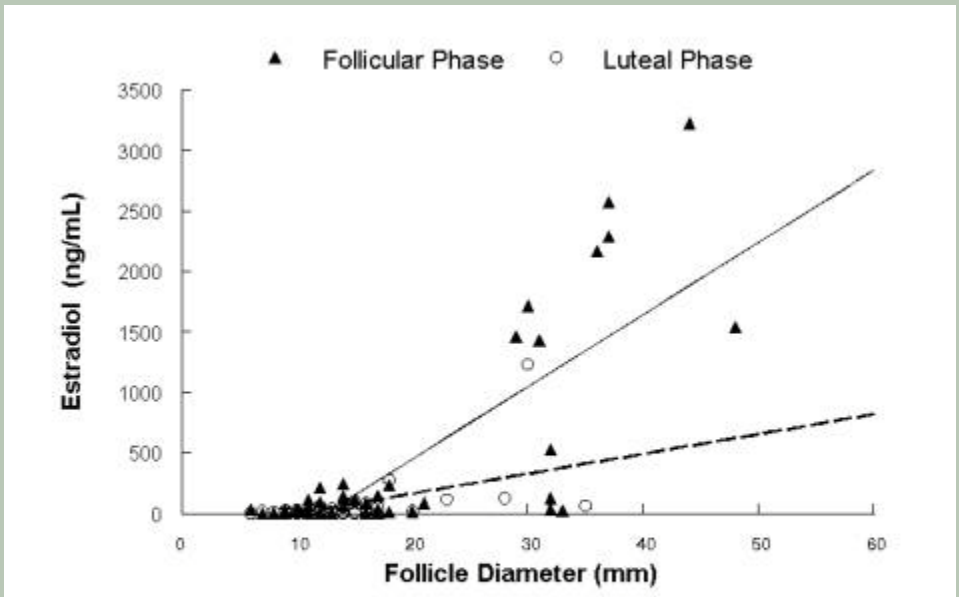

Figure 1. Linear regresson analysis of estradiol concentration in follicular fluid with follicle size during the follicular phase (—, n=54) or luteal phase (- - -, n=38) of mares.

Androstenedione concentration in follicular fluid averaged 80.1 ± 28.4 ng/mL and did not differ ($P>0.10$) between the follicular and luteal phases. However, androstenedione concentration

increased as follicle diameter increased (P<0.05). The fitted line of follicular fluid androstenedione concentrations for the combined follicular and luteal phases are in Figure 2.

Concentrations of IGF-I in follicular fluid during the follicular phase did not differ (P>0.10) from those during the luteal phase. However, IGF-I concentration increased as follicle diameter increased (P<0.05). The fitted line for the combined follicular and luteal phases of IGF-I are in Figure 3.

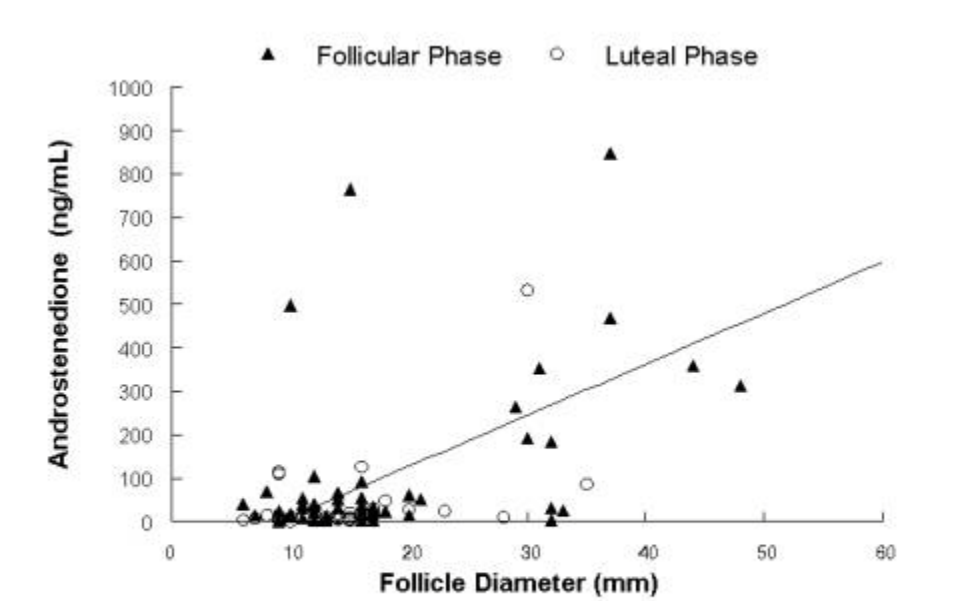

Figure 2. Linear regresson analysis of androsenedione concentration in follicular fluid with follicle size during the follicular and luteal phases (combined analysis, n=92) of mares.

Discussion

Previous findings are consistent with our observations that intrafollicular estradiol increases during follicular growth (Meinecke et al., 1987; King and Evans, 1988; Gerard and Monget, 1998; Goudet et al., 1999). Van Rensburg and Van Niekerk (1968), using 16 follicles, noted increases in follicular fluid estradiol concentrations with increases in diameter of follicles > 20 mm. Similarly, our results indicate that concentrations of estradiol do not dramatically increase until follicles reach > 25 mm in diameter. Gerard and Monget (1998) indicated that an increase in estradiol levels occur from early dominant (22-25 mm) follicles to late dominant (33-35 mm) follicles. Similarly, Belin and coworkers (2000) found that estradiol levels were lower in dominant follicles at emergence (approximately 20 mm in diameter) than at the end of follicular growth ($\geq$ 30 mm in diameter) or in preovulatory follicles. Aromatase activity dramatically increases from 20-24 mm follicles to 25-29 mm follicles at the end of the follicular phase (Goudet et al., 1999). In the mare, this increase in total IGF-I levels may act to increase the sensitivity of the follicle to gonadotropins, stimulating granulosa cell differentiation and follicular development.

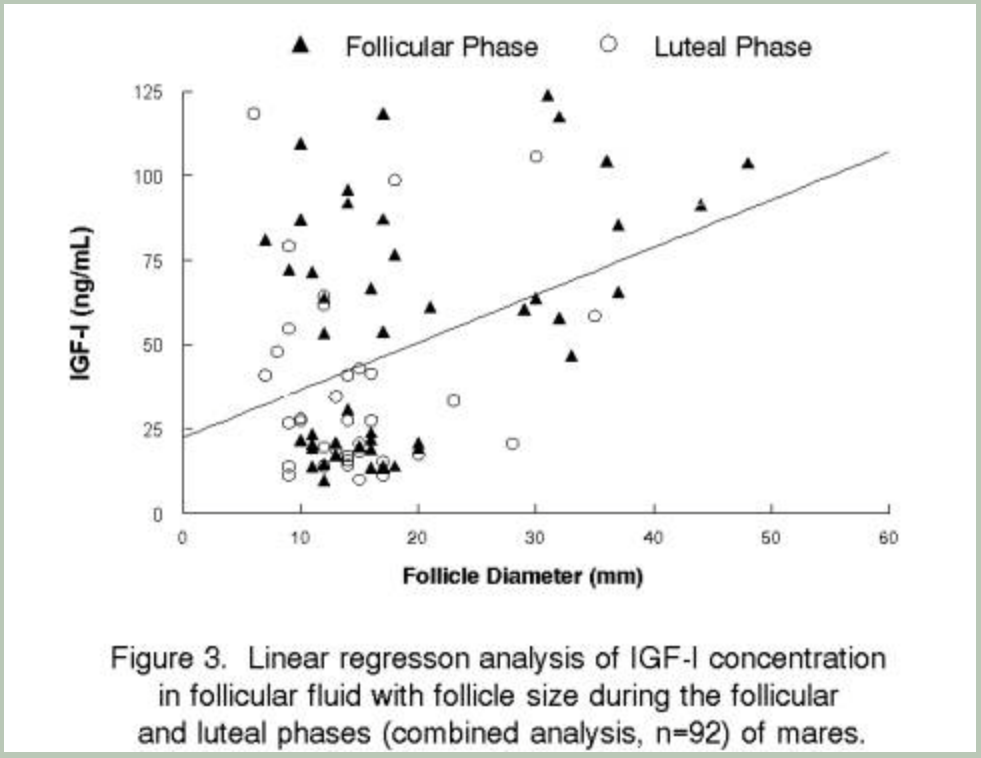

Figure 3. Linear regresson analysis of IGF-I concentration in follicular fluid with follicle size during the follicular and luteal phases (combined analysis, n=92) of mares.

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Acknowledgments

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