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Intrafollicular Concentrations of Steroid Hormones and $PGF_{2\alpha}$ in Relation to Follicular Development in the Mares during the Breeding Season

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The concentrations of androstenedione, estradiol-17β, progesterone and $PGF_{2\alpha}$ contained in the follicular fluid produced by the follicles in collected ovaries of mares that have had estrous phase during the breeding season were measured and analyzed the relation between the growth stage of follicles and the hormone levels in the follicular fluid. An ultrasonographic diagnostic instrument was used to measure the diameter of the follicles in order to categorize the follicles into three groups the following: 8 small follicles (from 1.0 to less than1.5 cm), 8 medium follicles (from 1.5 to less than 3.0 cm), and 8 large follicles (from 3.0 to 5.0 cm), respectively. The analysis of the follicular fluid in ovaries of estrous mares showed that the concentrations of androstenedione were significantly higher in the medium or large follicles than in the small follicles and the concentrations of estradiol-17β were significantly higher in larger follicles than in the small or medium follicles ($P<0.05$). The concentrations of progesterone and $PGF_{2\alpha}$, on the other hand, did not significantly vary regardless of follicluar size. In the follicles within the mare ovaries that have had estrous stage, the concentrations of the hormones related the ovulation, namely androstenedione and estradiol-17β, were higher with larger follicles.

Key words: *follicular fluid, mare, $PGF_{2\alpha}$ concentration, steroid hormones*

J. Equine Sci.
Vol. 19, No. 2
pp. 31–34, 2008

The breeding season of mares is artificially controlled in such a way that it begins in early February. While the estrous cycle of a mare is within the range of 20 to 24 days (mean: 23 days), the estrous phase in individual mares varies greatly, ranging from 2 to 12 days (mean: 7 days), which is characteristically longer than in cows. Dominant follicles are responsible for estrous induction, and grow approximately 3.5 mm per day until maturity at 5 to 7 days after the onset of estrus. Mature follicles are 45 to 60 mm in diameter just before ovulation in mares [9]. Ovulation occurs at 1.5 to 2 days before the disappearance of estrous signs [8]. Previous studies have found that the blood concentration of luteinizing hormone (LH), which induces ovulation, starts rising at 3 to 5 days before ovulation, stays at its peak level for 1 to 2 days following ovulation, then declines to the basal level by 5 days post-ovulation [5, 8].

Therefore, we collected the mare ovaries that were determined as being estrous phase during the breeding season at an abattoir. We then measured the level of hormones contained in the follicular fluid produced by the follicles within the collected ovaries from estrous mares, and analyzed the relationship between the growth stage of follicles and the steroid hormone and $PGF_{2\alpha}$ levels in the follicular fluid.

Ovaries were collected from 18 heavy draft mares that have had the breeding season (from April to June), including the Breton, Percheron, Belgian breeds and their crossbreed horses at Kumamoto-city abattoir, Japan. Ovaries that did not show any abnormalities

This article was accepted January 28, 2008
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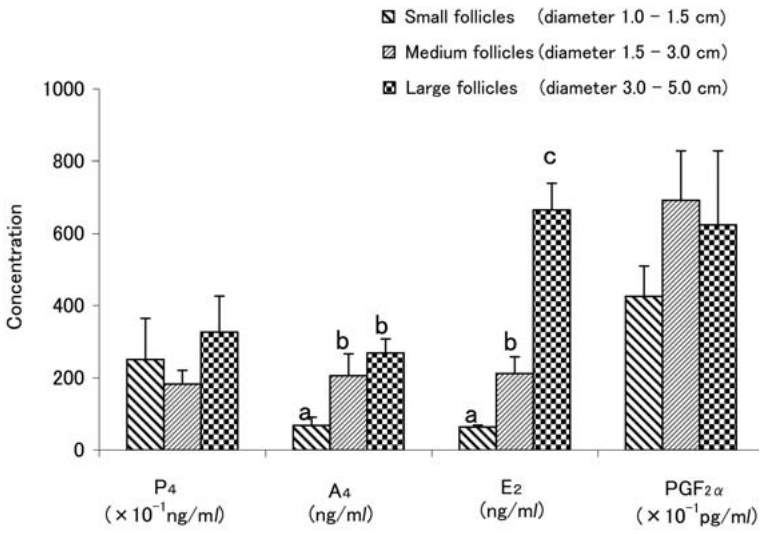

Fig. 1. Relationships between the follicular size and the hormone levels in the follicularfluid in mares with the onset of estrus (mean ± SE). P_4 : Progesterone, A_4 : Androstenedione, E_2 : Estradiol-17β, $PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$.
a, b, c: Values with different letters represent signifficantly difference ($P<0.05$).

upon megascopic examination were lightly rinsed with 1,000 m*l* of physiological saline solution supplemented with penicillin 100,000 IU and streptomycin 100 mg, according to Kojima's method [7]. Such ovaries were then preserved in cold physiological saline solution and transported into our laboratory. Sixteen of the collected ovaries were excluded from the study because of their appearance or the existence of either hemorrhaged follicle, sticky fluid follicle, atretic follicle and corpus hemorrhagicum or functional corpora lutea confirmed by the combination of the detection of follicular waves, degree of the opening of the ovulation fossa, and ultrasonotomogram. An ultrasonographic diagnostic instrument was used to measure the diameter of the follicles within the remaining 20 ovaries in order to categorize the follicles into three groups the following: small follicles (from 1.0 to less than 1.5 cm), medium follicles (from 1.5 to less than 3.0 cm), and large follicles (from 3.0 to 5.0 cm). From each category, eight follicles were randomly selected. A 21 G injection needle connected to a 50 m*l* injection syringe was used to puncture the side of the ovarian parenchyma and aspirate the follicular fluid. The collected follicular fluid was frozen at –20°C for preservation up to the time of hormone level analysis.

The concentrations of progesterone, androstenedione, estradiol-17β, and $PGF_{2\alpha}$ in the follicular fluid were measured with RIA according to Acosta, *et al.* [1], Kojima [7] and Wijayagunawardane, *et al.* [15]. In this procedure, the intra- and inter-assay variability of progesterone, androstenedione, estradiol-17β, and $PGF_{2\alpha}$ concentrations were 4.8 and 7.5%, 5.3 and 8.2%, 6.3 and 8.5%, and 8.7 and 13.1%, respectively.

ANOVA and Student's *t*-test were employed to analyze the concentrations of progesterone, androstenedione, estradiol-17β, and $PGF_{2\alpha}$ in the follicular fluid of the small, medium, and large follicles.

Figure 1 showed the concentrations of progesterone, androstenedione, estradiol-17β and $PGF_{2\alpha}$ in the follicular fluid of the small, medium, and large follicles. The progesterone concentrations in the follicular fluid did not significantly vary by follicular size. The mean androstenedione concentration in the follicular fluid of small follicles was 68.4 ± 22.7 ng/m*l* (mean ± SE), which was significantly lower ($P<0.05$) than those in the follicular fluid of the medium (206.1 ± 60.8 ng/m*l*) and large follicles (269.2 ± 38.7 ng/m*l*). The mean estradiol-17β concentration in the follicular fluid was 64.3 ± 5.0 ng/m*l* in the small follicles and clearly higher with the follicles of a greater diameter, reaching 212.2 ± 46.4 ng/m*l* in the medium follicles and 665.2 ±

74.1 ng/m*l* in the large follicles. The difference of the estradiol-17β concentrations among the three follicle groups was statistically significant ($P<0.05$). The mean $PGF_{2\alpha}$ concentrations in the follicular fluid of the small, medium, and large follicles were 42.6 ± 8.6, 69.2 ± 13.7, and 62.4 ± 20.5 pg/m*l*, respectively, showing no statistical difference among the three follicle groups.

It has been considered that blood estradiol-17β levels and urine estrone sulfate levels reach their peak at one to two days before ovulation in mares [5]. The present study demonstrated that the concentrations of androstenedione and estradiol-17β in the follicular fluid of large follicles are higher than those follicles in small or medium follicles. This finding was consistent with the report by Belin, *et al.* [2]. According to Watson and Sertich [13] and Watson, *et al.* [14], the testosterone levels in the follicular fluid of mature follicles was also high. In the present study, androstenedione concentration in normal large follicles was higher than that in small or medium follicles. Sirois, *et al.* [11] cultured the theca intra cells of the follicles within ovaries during the diestrous phase and observed no production of progesterone, androstenedione, or estradiol-17β, supporting our finding of the low hormone levels in the small follicles. Silberzahn, *et al.* [10] stated that the testosterone concentrations in the follicular fluid did not significantly differ between developing and atretic follicles. In the domesticated animals other than horses, $PGF_{2\alpha}$ levels are known to rise immediately before ovulation. Evans, *et al.* [3, 4] reported that such a phenomenon might be associated with the luteinization of the granulosa cells and ovulation. Ginther [6] indicated that, in mares, $PGF_{2\alpha}$ in the follicular fluid was produced by the granulosa cells and might be associated with ovulation although it did not directly impact the steroid production from the theca interna. In the present study, there was no significant difference in the $PGF_{2\alpha}$ levels among the follicular fluids collected from the small, medium, and large follicles. Such a result probably suggests that there were no follicles immediately before ovulation in any of the test ovaries in mares. In mature follicles, it has been considered that gonadotropic hormones act as survival factors whereas progesterone and androstenedione suppress follicular growth [12]. The present study showed that the estradiol-17β levels in the follicular fluid of large follicles were markedly high, indicating that the granulosa cells were progressively proliferating while their apoptosis was suppressed.

In summary, the present study revealed the following: In the follicles within the mare ovaries that have had estrous stage during the breeding season, the concentrations of the hormones related the ovulation, namely androstenedione and estradiol-17β, were higher with larger follicles.

Acknowledgment

The authors thank Professor Akio Miyamoto (Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan) for the teaching of RIA analysis of hormones. We thank the staff of the Kumamoto-city abattoir for their kind cooperation in collection of samples.

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