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Profiles of Reproductive Hormone in the Microminipig During the Normal Estrous Cycle

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Abstract. *We investigated changes in the peripheral plasma concentrations of ovarian steroids and gonadotropins throughout the estrous cycle of the Microminipig (MMpig), a novel experimental animal model. The mean (±standard error of the mean (SEM)) duration of estrous and the estrous cycle in seven animals was 66.0±5.2 h and 20.6±0.3 days, respectively. A luteinizing hormone (LH) surge was observed 16.5±6.5 h after the onset of estrous in six out of the seven animals. The LH peak and the duration of the LH surge were 14.0±4.5 ng/ml and 34.0±2.0 h, respectively. Plasma progesterone concentrations started to decline on Day 7 (Day 0 = the day of the LH peak) and increased from Day 4. Estradiol-17β levels increased from Day 3 and reached a maximum (37.0±1.6 pg/ml) on Day 0.75. Follicle-stimulating hormone (FSH) concentrations showed a first and second surge on Days 0 and 1.75. An inverse relationship between FSH and estradiol-17β concentrations was noted before the LH surge. The characteristics of estrous and the gonadotropin and ovarian steroid profiles throughout the estrous cycle of MMpigs are similar to those of domestic pigs and other miniature pig strains.*

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Pigs are an attractive animal model for the study of human diseases because of their physiological and anatomical similarities to humans (1-3). However, it is difficult to manage domestic pigs as laboratory animals because of their rapid growth and large mature body size. The miniature pig is as good a model for humans, as the commercial pig (3), with advantages in terms of convenience of management and handling. The Microminipig (MMpig), the smallest available miniature pig, was recently developed in Japan (4). The MMpig reaches approximately 10 kg body weight at 9 months of age and its fully mature weight is approximately 20 kg (4). Basic data on the biochemistry and physiology of novel experimental animals are important to determine whether these animals are suitable models for studies of human health. The general biochemistry and hematology (5-8) of MMpigs is similar to that of humans. Their utility as an experimental animal model, based on their biochemical and physiological similarities to humans, has been validated for the evaluation of atherosclerosis (9-11), proarrhythmias (12), sleep conditions (13), coagulation activity (5), skin toxicity (14) and genomic sequence (15).

In domestic animals, such as cattle and pigs, novel techniques for improving production efficiency have been developed based on basic physiology. Research into the reproductive physiology of animals is required to allow for successful breeding programs to produce a reliable supply of sufficient numbers of animals. However, the basic reproductive physiology of the MMpig, such as the normal estrous characteristics and basic gonadotropin and ovarian steroid profiles, has not been reported. The aim of this study was to clarify the characteristics of normal estrous and the basic profiles of gonadotropins and ovarian steroids throughout the estrous cycle in MMpigs.

Materials and Methods

Animals and estrous detection. Seven mature MMpig gilts were purchased from Fuji Micra Inc. (Shizuoka, Japan) and maintained at Kagoshima University in a dedicated, climate-controlled room with a temperature of 18-25˚C and humidity of 30-65%, with a 12 h light/dark cycle at the breeder's facility. MMpigs were kept in individual cages with water available *ad libitum* and were fed a commercial diet for domestic pigs (Kodakara 73, Marubeni Nissin Feed Co., Tokyo, Japan) once daily. Estrous detection was performed twice daily using a mature male MMpig. Estrous was defined as the period during which female animals demonstrated a standing response for the boar. For the purposes of this study, females were selected after having shown two or three estrous cycles. The mean ages and weights of the study animals were 24.1±4.5 months and 16.0±0.9 kg, respectively; mean±standard error of the mean (SEM). All protocols were approved by the Kagoshima University Ethics Committee of Animal Care and Experimentation (VM12001) and the research was performed according to the Institutional Guidelines for Animal Experiments and in compliance with the Japanese Law Concerning the Protection and Control of Animals (Law No. 105 and Notification No. 6).

Blood collection. Each gilt was fitted with a heparin-coated polyurethane catheter (CBAS-C70; Solomon Scientific, Plymouth Meeting, PA, USA) in the jugular vein at least 3 days before the experiment. Blood samples were collected daily beginning 10 days after the onset of estrous and continuing until 10 days after the onset of the subsequent estrous. Additionally, blood samples were collected at 6-h intervals from 18 days after the onset of estrous until the end of the subsequent estrous. Plasma was recovered after centrifugation of the blood samples and stored at −20˚C until analysis.

Hormone assay. Time-resolved fluoroimmunoassay (Tr-FIA) was used to measure plasma estradiol-17β, progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations. Plasma concentrations of estradiol-17β and progesterone were measured with a Tr-FIA kit (DELFIA Estradiol and Progesterone kits; PerkinElmer Japan, Yokohama, Japan) as previously reported (16). The intra-assay and inter-assay CVs were 8.1% and 8.1% for estradiol-17β and 10.0% and 9.1% for progesterone, respectively. Plasma concentrations of LH and FSH were determined using Tr-FIA methods previously described by Noguchi et al. (16) and Ohnuma et al. (17). The intra-assay and inter-assay CVs were 15.6% and 9.9% for LH and 16.0% and 8.4% for FSH, respectively.

Statistical analyses. The duration of the LH surge was considered the time from the onset to the end of the LH surge, as defined using a previously described method (18). All data were aligned relative to the LH peak on the basis of blood samples taken at 6-h intervals (Day 0=the day of the LH peak). The estrous cycle was divided into two periods: prior to the LH surge (Days 10 to 0) and after the LH surge (Days 0 to 8). Statistical analyses were performed using the general linear models procedure in statistical analysis software (SAS) (link or supplier with address). Data pertaining to the hormonal profiles were subjected to repeated measures analysis of variance (ANOVA) (19). When a significant effect was detected with ANOVA, the significance of the difference between the means was determined using the Tukey's test. Correlations between estradiol-17β and FSH concentrations were also assessed. In our data, the estradiol-17β concentrations between Days 2 and 3 were less than 4 pg/ml in four MMpigs and greater than 8 pg/ml in three MMpigs. In domestic pigs, plasma estradiol-17β concentrations increased to greater than 7 pg/ml when large antral follicles (over 6 mm in a diameter) are present on the ovaries (20). Thus, the hormonal data from Days 1 to 3 were divided into two groups, with (n=3) or without (n=4) increased estradiol-17β concentrations and the differences between the two means were tested for significance using the Student's *t*-test. A p-value of <0.05 was considered statistically significant.

Results

The mean durations (±SEM) of estrous and the estrous cycle, taken from observations of 18 estrous cycles, were 66.0±5.2 h and 20.6±0.3 days, respectively. An LH surge was observed at 16.0±6.5 h after the onset of estrous in six out of the seven animals. The peak and duration of the LH surge were 14.0±4.5 ng/ml and 34.0±2.0 h, respectively.

Steroid hormone and gonadotropin concentrations in MMpigs throughout the estrous cycle are shown in Figure 1. Peripheral progesterone concentrations were significantly lower ($p<0.05$) on Day 7 than on Days 8 to 10, and lowest on Day 5. Progesterone concentrations were then maintained at less than 1 ng/ml through Day 1, until rising to over 1 ng/ml on Day 1.9±0.1. Progesterone levels increased from Day 4 and plateaued on Day 6. Estradiol-17β concentrations were significantly greater ($p<0.05$) on Day 3 than between Days 10 and 4. They continued to increase and reached a maximum of 37.0±1.6 pg/ml on Day 0.75. Concentrations of estradiol-17β started to decrease ($p<0.05$) from Day 0.25 relative to the concentrations between Days 1.25 and 0.5. There was no significant increase in plasma estradiol-17β concentrations between Days 0.25 and 8.

FSH levels were significantly less ($p<0.05$) between Days 3 and 1 (excluding Day 2.5) than between Days 9 and 7. They reached a small, but significant, peak (2.9±0.4 ng/ml) on Day 0. The highest mean FSH concentration (4.8±0.6 ng/ml) was on Day 1.75. This concentration declined significantly by Day 3 and then showed no significant changes after Day 3. FSH and estradiol-17β concentrations were inversely-correlated between Days 10 and 0 ($r=-0.35$, $p<0.01$); however, there was no significant correlation between the two hormones from Days 0 to 8 ($r=0.07$, $p\geq0.1$). The LH concentration clearly peaked ($p<0.05$) on Day 0 with no other significant changes throughout the rest of the cycle.

The estradiol-17β concentrations of three animals on Day 2 tended to be high ($p=0.06$) relative to the other four animals (Table I). There were also no significant differences in progesterone, FSH and LH concentrations between animals with or without a small estradiol-17β increase after the LH peak.

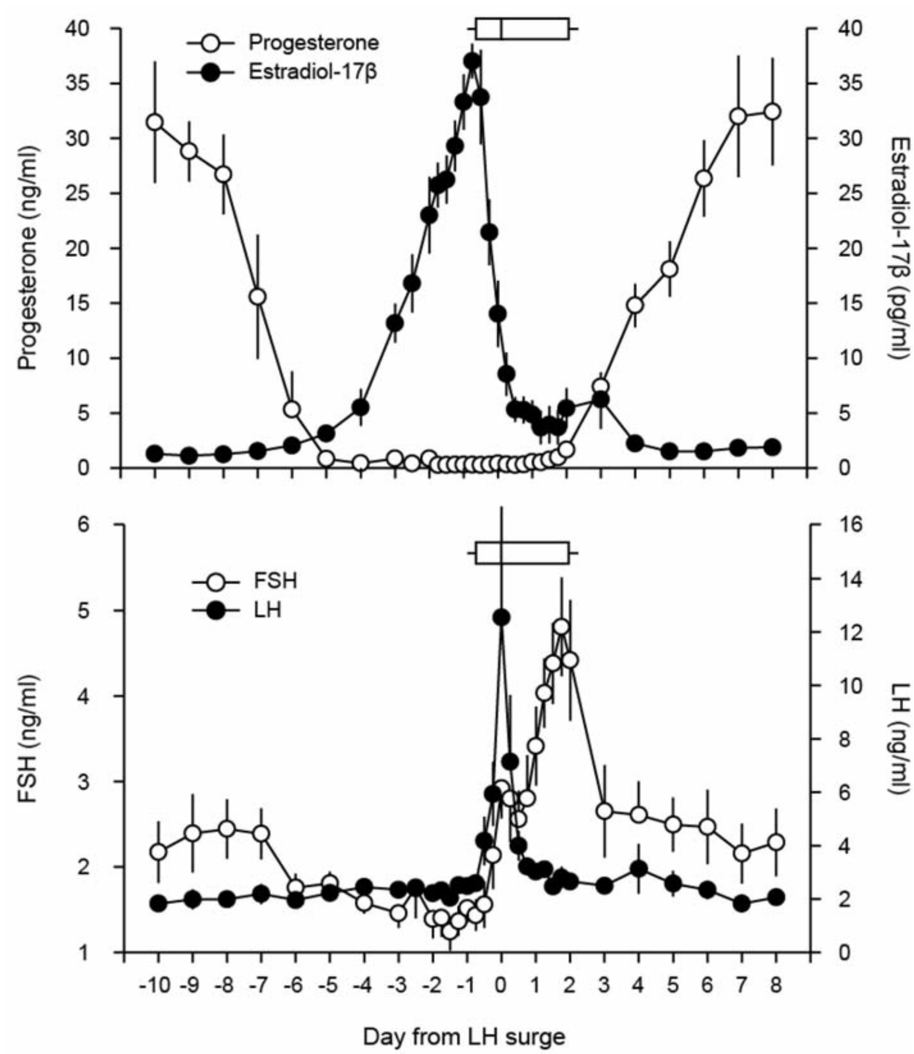

Figure 1. *Plasma concentrations of (a) progesterone and estradiol-17β, (b) FSH and LH in female MMpigs (n=7) during a natural estrous cycle. Hormonal data are aligned relative to detection of the LH surge (LH peak=Day 0). The bars indicate the mean number of days from the LH surge until estrous. Values are means±SEM.*

Discussion

Our data show the general estrous length and interval in female MMpigs. The duration of estrous in MMpigs tended to be longer than in domestic pigs (12-88 h) (21-24) but was similar between MMpigs and other miniature pigs (1.9-3.1 days) (25-27). The estrous interval in female MMpigs corresponded to previous reports on commercial pigs (21.3 days) (20) and other miniature pigs (18.3-22.6 days) (25, 28, 29).

The plasma progesterone concentration profile of MMpigs was similar to that of commercial pigs (20) and other miniature pig strains (25, 28). Progesterone levels in the MMpigs started to decrease from 7 days before the LH surge, which is similar to the progesterone concentration decrease observed in commercial pigs (20). We found that plasma progesterone concentrations became greater than 1 ng/ml at 36–54 h after the LH surge, while they subsequently increased. Peripheral progesterone concentrations in domestic pigs rise to greater than 1 ng/ml at 48 h after the LH peak and 24 h after ovulation (20, 21). Ovulation occurs 24-48 h after the LH peak in commercial pigs (20, 21, 30) or after the onset of estrous in another strain of miniature pigs (25). Progesterone levels can be used as an index of the development and luteolysis of the corpus luteum (CL) because CL diameter is strongly correlated with plasma progesterone concentrations in cows (31), goats (32) and pigs (20). Our data and previous reports suggest that, similar to domestic pigs, ovulation and CL formation in MMpigs may occur within 48 h after the LH peak.

Table I. *Hormone concentrations in animals with or without a small estradiol-17β peak after the LH surge.*

		With small peak	Without small peak	*p*-Value
n		3	4	–
Progesterone (ng/ml)	Day 1	0.8±0.3	0.4±0.1	0.17
	Day 2	2.2±0.6	1.3±0.3	0.22
	Day 3	8.5±2.2	6.6±1.7	0.51
Estradiol-17β (pg/ml)	Day 1	5.2±2.9	4.6±2.5	0.84
	Day 2	10.0±2.1	2.0±0.2	0.06
	Day 3	11.9±4.7	2.0±0.8	0.17
FSH (ng/ml)	Day 1	4.0±0.5	3.0±1.4	0.35
	Day 2	3.4±1.2	5.1±0.8	0.27
	Day 3	2.6±0.9	2.7±0.8	0.94
LH (ng/ml)	Day 1	3.0±0.5	3.0±0.2	0.98
	Day 2	2.7±0.4	2.6±0.3	0.89
	Day 3	2.9±0.5	2.2±0.2	0.21

Day 0 was defined as the day of the LH peak. Values are presented as means±SEM.

Although estradiol-17β levels before the LH surge are similar in the MMpigs and other pigs (25, 27, 28), the estradiol-17β profile of the MMpigs after the LH surge was similar to that of other miniature pig strains (25) but not domestic pigs (20). Estradiol-17β concentrations in three of our MMPigs showed small peaks 2 and 3 days after the LH surge but these were not detected in the other four MMpigs. Estradiol-17β is produced by the granulosa cells of large, mature follicles (33) and changes in peripheral plasma estradiol-17β concentrations are associated with the development of mature follicles (20). Our results, and those of previous reports, suggest that large ovarian follicles may develop after the LH surge in some MMpigs. Increased estradiol-17β following the first follicular wave, stimulated by gonadotropin secretion, has been observed 2 or 3 days after ovulation in cows (31, 34) and goats (32). In domestic sows, the number of small follicles (3-5 mm in diameter) and the inhibin concentrations increase after ovulation but a tendency for increased estradiol-17β levels, associated with the development of large follicles during the early luteal phase, has not been reported (20). Follicular development and maturation is regulated by FSH and LH stimulation. In heifers (35), the growth of the first dominant follicle is mainly influenced by the frequency of the LH pulses and the mean concentrations of LH. There were no differences in the gonadotropin concentrations measured daily between groups with and without an estradiol-17β increase after the LH surge in this study; however, follicular development and maturation may be related to the pulsatile secretion of gonadotropins. The association between folliculogenesis and hormonal changes during the early luteal phase in MMpigs remains unclear, thus additional experiments may be required.

Plasma concentrations of progesterone during the luteal phase and estradiol-17β levels before the LH surge differed between MMpigs and domestic pigs, even when the same assay methods were used (20). The progesterone concentration plateau after the LH surge in MMpigs (26.4 ng/ml) was higher than in domestic pigs (17.6 ng/ml) (20). The peak estradiol-17β concentration in MMpigs (37.0 pg/ml) was also high compared with that seen in domestic pigs (20.8 pg/ml) (20). Post-mortem examinations revealed seven large follicles (over 5 mm in a diameter) at the onset of estrous (n=1), three and seven functional CLs (over 6 mm in a diameter) at 7 days after the onset of estrous (n=2) and eight luteolytic CLs (under 4 mm in a diameter) at 17 days after the onset of estrous (n=1) in MMpigs (unpublished data). These data suggest that approximately five follicles may develop and subsequently ovulate during normal cycling in MMpigs. It has been previously reported (20) that the number of mature follicles in domestic pigs, weighing from 175 to 240 kg, ranges from 10 to 17. Our results raise the possibility that the higher concentrations of steroid hormones in MMpigs may be caused by a higher number of follicles and CLs per kg of body weight in MMpigs compared with domestic pigs.

The LH and FSH profiles of MMpigs were also similar to those of commercial pigs (20). Although the peak LH concentrations (7.5-35.7 ng/ml) in MMpigs seem higher than the LH concentrations (4.4-9.0 ng/ml) in commercial pigs (16, 20, 21, 30), the duration of the LH surge (34.0 h) in MMpigs was consistent with that previously reported in domestic pigs (27.0-35.2 h) (16, 20, 21, 30). The interval from the onset of estrous to the LH surge in six MMpigs was similar to that previously reported in domestic pigs (16, 20, 21) and in another strain of miniature pigs (25).

There was an inverse relationship between the FSH and estradiol-17β levels during the late luteal and follicular phases in MMpigs, which was similar to that seen in domestic pigs ($r=-0.38$) (20) and cows ($r=-0.42$) (31). However, there was no relationship between plasma FSH and estradiol-17β levels after the LH surge in either all of the animals or in the animals in which estradiol-17β concentrations increased after the LH surge. These results suggest that plasma FSH concentrations are inversely related to estradiol-17β concentrations during the late luteal and follicular phases; however, as in the domestic pig and cow, estradiol-17β seems to have only a weak regulatory effect on FSH during the early luteal phase in MMpigs.

In conclusion, our results indicate that the characteristics of estrous and the gonadotropin and ovarian steroid profiles throughout the estrous cycle in MMpigs are similar to those of domestic pigs and other miniature pig strains. The knowledge of the basic reproductive physiology of female MMpigs gained from our study may improve production efficiency and aid in the establishment of new reproductive techniques, such as estrous/ovulation synchronization and superovulation in MMpigs.

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Conflicts of Interest

There are no conflicts of interest in this report.

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