Serum Testosterone and Progesterone Concentrations in Lanyu Pigs

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ABSTRACT

In response to the policy to select laboratory minipigs for medical research, native miniature pigs were introduced from Lanyu Island in 1980 by the Taitung Animal Propagation Station of Livestock Research Institute. After almost 30 years of conservation and selection projects, several inbred herds have been registered as new minipig breeds for biomedical research. However, since natural mating has been the breeding method facilitated in the past, no records were kept on the reproductive physiology of the pigs. The purpose of this study is to analyze the changes in boars as they reach sexual maturity and the endocrine status of sows during estrous cycle. The experiment is divided into two parts. The first part of the study is to observe the changes of serum testosterone(T) concentrations among boars, mainly by collecting boars of 8 different growth stages at 0, 4, 8, 12, 16, 20, 24, and 28 weeks to measure their body weight, testicle weight and serum testosterone concentrations. The second part of the study is to observe the changes in progesterone(P₄) concentrations in sows during estrous cycle, mainly by measuring the serum progesterone of 2 multiparous sows and 3 first-litter sows. The frequency of serum sampling is twice a day for a total of 40 days. The results show that the boars’ testosterone concentration are lower than 0.1 ng/ml at 0 to 16 weeks, but increases to 1.97 ng/ml at 16 to 20 weeks, and then to 5.25 ng/ml at 20 to 28 weeks. The estrous cycle of the five sows is 23.2±1.6 days. The first multiparous sow was observed to have an increase in progesterone concentration after weaning, with the progesterone concentration peaking (48.8 ng/ml)14 days after weaning. In the second sow, the progesterone concentration peaked (40.7 ng/ml) 1 day after weaning. The other three first-litter sows peaked in their progesterone concentrations (47.6 ng/ml, 50 ng/ml and 38 ng/ml) 4 to 6 days after weaning. In conclusion of the study results, it should take more than 20 weeks for male Lanyu pigs to reach sexual maturation, while the estrous cycle of the female Lanyu pigs are less stable despite the fact that their estrous cycle is similar with common pigs. This preliminary study should be beneficial to future studies in the area of reproductive physiology of Lanyu pigs.
**INTRODUCTION**

The miniature pig has been developed as an animal model for biomedical research, because they have a number of characteristics in common with human, such as a non-seasonal cycle estrus. However, neither Yucatan nor Götingen miniature pig are major laboratory miniature pig in Taiwan, whereas the Lanyu miniature pig is. The Lanyu miniature pigs were imported from Lanyu Island by Livestock Research Inst., C.O.A. for the policy that developing laboratory swine for medical research in 1980 and the conservation and breeding projects have being held at Taitung Animal Propagation Station since then. Several new miniature breeds were created and registered in recent years, and the application in various biomedical studies increased annually. However, the reproductive prolificacy of miniature pig generally has not compared favorably with that of another standard-sized counterpart. In 1980, Taitung Animal Propagation Station (TAPS) initiated a breeding program to develop an inbred strain of miniature pig that the mean number of ovarian follicles 9.3±1.8 and mean litter size of 5.2 offspring / female. The mode of natural mating in the past has not been appropriate now for the biomedical miniature pig production. The aim of this project is to establish the testis weight and testosterone of male Lanyu miniature pig and progesterone cycle of female Lanyu miniature pig, and collect basic data.

**MATERIALS AND METHODS**

**Animals**

20 male Lanyu miniature pig, at 1 day and 4, 8, 12, 16, 20 and 24 weeks of age. Five multiparous Lanyu miniature pig were housed together in a partly shaded outdoor room, animal ranged in weight from 50 to 80 kg and in age from 11 to 32 months, multiparous Lanyu miniature pig has produced a mean of 1.4 ± 0.9(range 1 to 3) litters before the study. Use TLRI commercial standard-sized diet was fed twice daily. All animals were offspring of an inbreeding program that originated with four miniature boars and sixteen miniature sows. Blood sample were taken between 09:00 and 10:00 directly from the *vena cava* with a syringe, blood was kept at room temperature for 15 min, and serum sample were obtained by centrifugation (4°C, 3,500rpm, 15 min) and the blood serum samples were stored at -20°C until the assay.
Hormone assays
Serum testosterone and progesterone concentrations were assayed by the method of Wu (1989).

Results

Figure 1. shows the Lanyu boars have a positive weight gain of 1kg per week, and reaches 30 kg at 28 weeks. The testicle weight increases with the body weight, and reaches 56g at 20 weeks. And the blood testosterone concentrations collected at 8 different stages at 0, 4, 8, 12, 16, 20, 24 and 28 weeks, which indicates the testosterone concentrations are lower than 0.1 ng/ml at 0 to 16 weeks, but increases to 1.97 ng/ml at 16 to 20 weeks, then further increases to 5.25 ng/ml at 20 to 28 weeks. This explains the fact that miniature boars reaches sexual maturity at around 5 months. However, we advise against breeding at this point, and suggest pen mating beyond 6 months of age. Our mating method is selecting boars that have reached a weight of 25-30 kg at 6 months of age as alternate boars. The sows selected for insemination are either alternate sows or first-litter sows in order to prevent the multiparous sow from attacking the alternate boar due to large differences in body size.

The 5 sows from which blood samples were collected, with the day of weaning as day 0 for blood sampling. The frequency of blood sampling is once every 2 days, with a total of 40 days. Figure 2-1 is the cycle change of pig 1. The sow’s progesterone concentration increased 4 days post weaning, peaked on day 14 (48.8 ng/ml) and day 36 (46.6 ng/ml). The sow’s progesterone concentration
was at its lowest on day 2 (4.5 ng/ml) and day 22 (4.3 ng/ml). This indicates the estrus cycle of pig 1 is 22 days. From day 6 to day 8 the sow demonstrated clear estrus signs, indicating the Lanyu pig should reach estrus 6 to 8 days post weaning, its estrus characteristics similar to regular-sized pigs. Figure 2-2 is the cycle change of pig 2. The sow’s progesterone concentration peaked on day 2 (40.7 ng/ml) and day 24 (41.2 ng/ml) post weaning. The sow’s progesterone concentration was at its lowest on day 6 (1.9ng/ml) and day 28 (1.6ng/ml). This indicates the estrus cycle of pig 2 is 22 days. However, the sow had a high progesterone concentration on weaning day, indicating that it has reached estrus while still lactating at the farrowing house. No outward estrus signs were observed in pig 2, indicating the possibility of a silent estrus in pig 2. According to sow reproduction data from the breeding center, the reproductive performance of pig 2 did not meet general expectations. Further discussion and analysis will be necessary to reveal the cause in detail. Figure 2-3 is the cycle change of pig 3. The sow’s progesterone concentration peaked on day 4 (47.6 ng/ml) and day 26 (44.3 ng/ml) post weaning. The sow’s progesterone concentration was at its lowest on day 16 (3.1ng/ml) and day 40 (4.4ng/ml). This indicates the estrus cycle of pig 3 is 22 days. However, the
sow had a high progesterone concentration on the 4th day post weaning, indicating that it has reached estrus while still lactating at the farrowing house. From day 18 to day 20 the sow demonstrated clear estrus signs. Since pig 3 is a first-litter sow, initially it is believed that what caused the sow to experience estrus at the farrowing house might be due to an inadequate number of litter size. Further discussion and analysis will be necessary to reveal the cause in detail. Figure 2-4 is the cycle change of pig 3. The sow’s progesterone concentration peaked on day 6 (50 ng/ml) and day 28 (48.3 ng/ml) post weaning. The sow’s progesterone concentration was at its lowest on day 20 (2.6 ng/ml) and day 40 (5.6 ng/ml). This indicates the estrus cycle of pig 4 is 22 days. However, the sow had a high progesterone concentration on the 6th day post weaning, indicating that it has reached estrus while still lactating at the farrowing house. From day 18 to day 20 the sow demonstrated clear estrus signs. Pig 4 is a first-litter sow, pig 4 and pig 3 are sister sows from the same litter that demonstrated similar estrus cycle patterns. Figure 2-5 is the cycle change of pig 5. The sow’s progesterone concentration peaked on day 4 (38.2 ng/ml) and day 22 (46.0 ng/ml) post weaning. The sow’s progesterone concentration was at its lowest on day 12 (1.7 ng/ml) and day 38 (2.2 ng/ml). This indicates the estrus cycle of pig 5 is 18 days. However, the sow had a high progesterone concentration on the 4th day post weaning, indicating that it has reached estrus while still lactating at the farrowing house. From day 14 to day 16 the sow demonstrated clear estrus signs. Pig 5 is a first-litter sow.

Discussion

In Taiwan, previous research on Lanyu pig tend to focus on the facilitation of conservation plans and the promoting of biomedicine. Fewer research were conducted on the reproduction performance of the boars and sows. The reproduction performance of Lanyu sows has long been decided by litter size, and not much discussion has been conducted on the sow’s physiological endocrinal changes. By looking into the endocrinal changes of the sows, this study will be useful in future research on the reproduction physiology of the Lanyu pigs. Therefore, we have concluded the following points for discussion: 1. Miniature boars reaches sexual maturity at 5 months (with a body weight of 20-25kg), their testicle weight increases at 16 weeks (with a body weight of 16-18kg). At the same time, there is a significant increase in testosterone concentrations, indicating that testosterone concentrations increase with testicle weight. But usually breeding takes place after the pigs reach 6 months of age (with a body weight of 25-30kg).
Compared with common pigs, miniature pigs reach sexual maturity earlier, this may have to do with an earlier secretion of testosterone. Since miniature pig is considered as an indigenous pig variety, its characteristics include early sexual maturation and good acceptance toward roughage feed. It could be the course of evolution to allow miniature pigs reach sexual maturation sooner to shorten the time for reproduction.

Based on earlier findings on farrowing sows, most Lanyu pigs reach estrus 1-2 weeks after weaning. This data is in accordance to this type of mating method. Since sows reproduce mainly by the natural mating method, it is difficult to effectively control their gestating and farrowing cycles, thus resulting in flawed management. By understanding the exact estrus cycle post parturition should help adjust the sow parturition and population control. The first 2 sows (pig 1 and pig 2) are older Lanyu pigs with less consistent estrus cycles post parturition. Pig 1 reached estrus 6 to 8 days post weaning. However, pig 2 reached estrus near the end of lactating. The early estrus indicates the sow had reproductive problems. Pig 3, 4, and 5 are younger Lanyu sows that demonstrated similar cases of reaching estrus in the farrowing house. Since this experiment did not monitor LH and E2, we cannot thoroughly understand the physiological endocrinal changes of the sow. The present study P₄ concentration increased 48 hours after LH surged maintained at high concentrations for 3 to 10n days, then decreased on day 19. The aforementioned data are evidence that Taiwan’s miniature Lanyu pig has progesterone secretion cycles similar to that of other miniature pigs, except the estrus cycle among the sows being less consistent. These questions and solving methods will be left for future research.