Changes in Serum Steroid Levels in Female Red Pandas

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ABSTRACT

Serum was collected from five female adult red pandas for 23 consecutive times during their estrus and gestation periods. The collected serum was analyzed through a radioimmunoassay to examine changes in estradiol (E2) and progesterone (P) content during the reproductive period of the red pandas. The results revealed the following: (1) When the female red pandas entered their estrus periods, the serum E2 content increased rapidly and peaked several times. Only one peak was consistent with mating behavior. The range of the estrus period length was 14–48 days, with the average length being 28.26 ± 13.24 days (n = 5). Peaks in serum E2 content were observed during the gestation period of pregnant red pandas and during the luteal phase of nonpregnant red pandas. (2) The serum P content increased during the gestation period of pregnant red pandas and luteal phase of nonpregnant red pandas. The P content started to increase after mating. Accordingly, we can deduce the following: (1) multiple estrus periods occur in red pandas during the reproductive season; (2) follicle development still occurs in the gestation period and luteal phase; (3) no evidence of delayed embryo implantation was found in captive breeding red pandas in Fuzhou, China, according to the change trend of the P content.

Keywords: *Delayed implantation, Estradiol, Estrus period, Gestation period, Luteal phase, Progesterone, Red panda.*

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Highlights of this paper

- Developing artificially bred species of red panda, improving research on the reproductive characteristics of the captive population of the species are highly vital toward increasing the species' propagation and reducing the capture of wild species.
- Only one peak of the serum estradiol content was consistent with mating behavior. The range of the estrus period length was 14–48 days, with the average length being 28.26 ± 13.24 days (n = 5).
- The serum progesterone content increased during the gestation period of pregnant red pandas and luteal phase of nonpregnant red pandas.

1. INTRODUCTION

The red panda (*Ailurus fulgens*) is native to the Himalayas and Hengduan Mountain Range. It is endemic in Asia [1] and has been classified as endangered by the Convention on International Trade in Endangered Species (CITES). The red panda is listed in Appendix I of the CITES catalog and classified as a protected class II wildlife species in China. Therefore, developing artificially bred species of red panda, improving research on the reproductive characteristics of the captive population of the species are highly vital toward increasing the species' propagation and reducing the capture of wild species. The red panda has a seasonal reproductive cycle, and both females and males reach sexual maturation at the age of 18–20 months.

The estrus period of the red panda occurs between mid-January and mid-March. An estrus period for female red pandas lasts for 12–36 hours, and two to three mating processes occur during this period. The red panda's gestation period is between 114 and 145 days, and its delivery period is between June and July [2, 3].

Gonadal hormones directly reflect mating activity in mammals. Accordingly, studying the regulatory process of gonadal hormones elucidates the periodic breeding mechanism of a specific mammal [4]. Thus far, only a few studies have reported the pattern of changes in the gonadal hormones of red pandas during their reproductive period, and such studies have mostly focused on measuring changes in sex hormone content in the urine and feces of animals Spanner, et al. [5]; Li, et al. [6]; Li, et al. [7]; Hu, et al. [8]; Xia [9]. Yunfang, et al. [10] reported on changes in the serum gonadal hormone content of red pandas during the reproductive period, but the serum sample size was small. To further investigate the pattern of changes in estradiol (E_2) and progesterone (P) content in captive red pandas, understand ovarian activity in red pandas during the estrus and gestation periods, and review the reproductive characteristics of the species, the present study examined the changes in serum E_2 and P content and the mating behaviors during the reproductive period of five female adult red pandas from December 2005 to July 2006.

2. MATERIALS AND METHODOLOGY

2.1 Conditions of Study Site

The Fuzhou Giant Panda Research Center is located on Dameng Mountain in Fuzhou City. The city has a subtropical marine climate, and the mean annual temperature is 19.6°C. The mean temperature is 10.5°C in January and 28.6°C in July. In addition, the highest annual temperature is more than 39°C, whereas the lowest annual temperature is 0°C. The annual relative humidity is 77%. The average annual precipitation is 1342.5 mm, with the average annual precipitation days being between 130 and 170 days. Finally, estimates of the average annual sunshine hours are between 1700 and 1980 hours.

2.2. Animals Testing

Five female adult red pandas were provided by the Fuzhou Giant Panda Research Center, and they were given numerical designations, namely red pandas 1–5. In November 2005, a physical examination revealed that the five

red pandas were in normal condition. Hematology and biochemical examination results were also normal. All red pandas in this study were raised in the same pen, and they lived with four male adult red pandas. The pen had a 60-m^2 cement floor and several wooden perches. The indoor exercise ground had a 100-m^2 grassland area, and there were several climbable trees and dummy mountains.

2.3. Blood Sample Collection

The red pandas had more than 2a of artificial training. They were trapped in a cage after using food to tempt them to enter the cage. Blood was collected from the vena cephalica of the red pandas without anesthesia. Only 2 mL of blood was collected each time per panda, and the blood was injected into a Vacutainer tube with coagulant (thromboplastin) and transported back to the laboratory within 30 minutes.

Centrifugation was conducted at 3000 rpm to separate serum (1.5 mL) into a polyethylene centrifuge tube. The tube was then sealed, marked, and placed in the refrigerator at -20°C until testing.

During the estrus and gestation periods, blood was collected from each red panda for a total of 23 times.

2.4. Animal Observation

The observation period was from December 1 each year until April 1 of the following year. The mating behaviors of the red pandas were observed and recorded from 8:00 to 10:00 a.m. and from 3:00 to 4:00 p.m. each day.

2.5. Hormone Measurement

E_2 and P radioimmunoassay kits were purchased from Beijing North Institute of Biological Technology. A GC-1200r radioimmunoassay counter was purchased from ANHUI USTC Zonkia Scientific Instruments Co., Ltd. The technical specifications of the test kit are shown as follows: (1) measuring ranges for E_2 and P of 5–4000 pg mL^{-1} and 0.2–100 ng mL^{-1}, respectively; (2) sensitivity levels for E_2 and P, <2 pg mL^{-1} and <2 ng mL^{-1}, respectively; (3) for precision, E_2 within-batch variation was < 10% and between-batch variation < 15%, and for P, within-batch variation < 10% and between-batch variation < 15%; and (4) average recovery rates for E_2 and P were 116.2% and 85.4%, respectively.

3. RESULTS AND ANALYSIS

Red panda 1 engaged in natural mating on February 8 and 9 and gave birth to an infant red panda after 163 days. The changes in its serum E_2 and P content are presented in Figure 1. The E_2 content peaked at 462.98 pg mL^{-1} on January 26 and 645.05 pg mL^{-1} on February 9. The peak value dropped rapidly following mating, and another peak value (456.35 pg mL^{-1}) was observed on March 15. The E_2 concentration remained above 300 pg mL^{-1} from March 15 to May 11 but subsequently dropped to below 100 pg mL^{-1} and remained at this new level. The P content remained at a low level before the estrus period and increased on February 8, but it dropped subsequently. The P content increased gradually on March 2 and peaked on March 15 (1.03 ng mL^{-1}), and then dropped again subsequently. After June 15, the P content remained at base level (lower than 0.1 ng mL^{-1}).

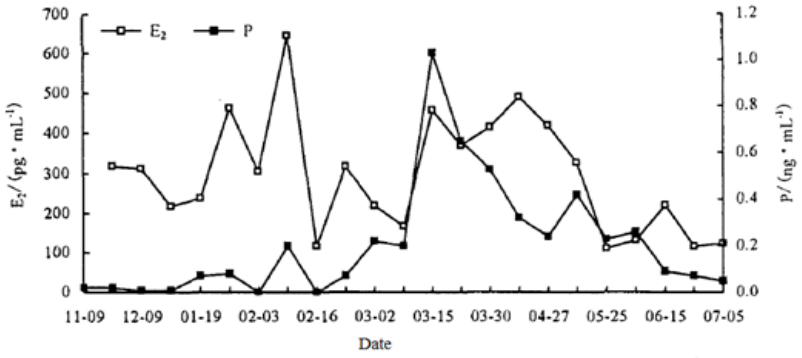

Figure-1. Serum concentration of estradiol and progesterone in animal 1.

Red panda 2 engaged in natural mating on February 20 and gave birth to an infant red panda after 148 days. The changes in serum E_2 and P content are illustrated in Figure 2. The E_2 content peaked on December 19 (585.3 pg mL^{-1}), January 26 (539.40 pg mL^{-1}), and February 20 (1067.58 pg mL^{-1}). The E_2 content dropped rapidly following the mating, but it increased on April 13 and reached 1051.59 pg mL^{-1}. The concentration dropped subsequently, and the lowest level was noted on June 5. The P content remained at a low level before the estrus period, but began to increase after mating and surpassed 0.1 ng mL^{-1} on June 15. The P content remained at base level after June 25 (below 0.1 ng mL^{-1}).

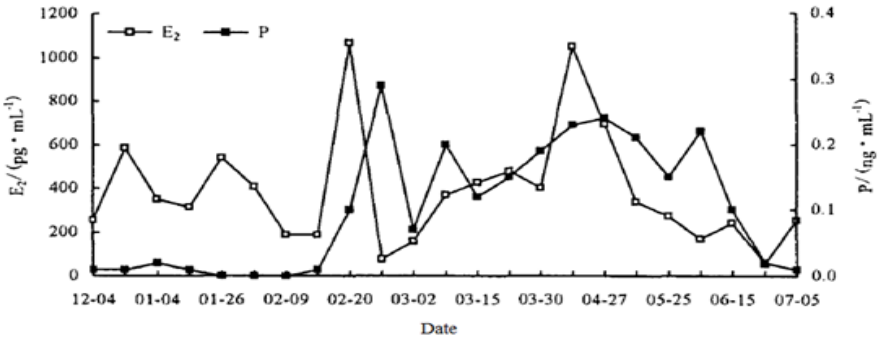

Figure-2. Serum concentration of estradiol and progesterone in animal 2.

Red panda 3 exhibited estrus behavior, but did not demonstrate mating behavior or give birth. The changes in serum E_2 and P content are shown in Figure 3. The E_2 content peaked on December 4 (224.6 pg mL^{-1}), February 16 (218.0 pg mL^{-1}), and March 23 (247.89 pg mL^{-1}). Another relatively high E_2 level was observed on April 27 (203.95 pg mL^{-1}). The P content increased after March 23 and peaked on April 13 (0.35 ng mL^{-1}), and it was undetected from April 27 to July 5.

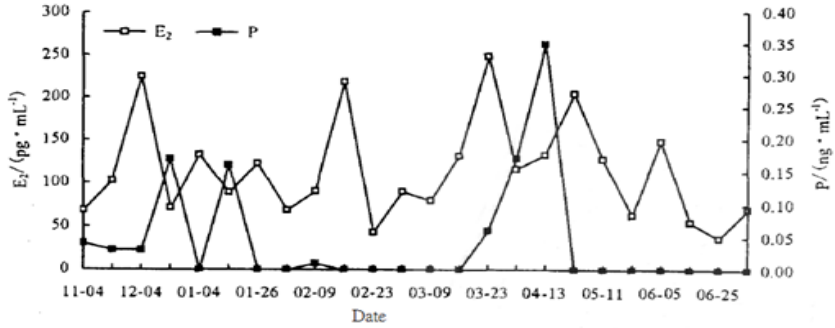

Figure-3. Serum concentration of estradiol and progesterone in animal 3.

Red panda 4 did not exhibit estrus or mating behavior, or give birth. The changes in serum E_2 and P content are illustrated in Figure 4. The E_2 content peaked on December 4 (1261.69 pg mL^{-1}), February 3 (1199.31 pg mL^{-1}), March 23 (1343.32 pg mL^{-1}), and April 13 (2689.5 pg mL^{-1}). Another peak was observed on May 11 (1664.75 pg mL^{-1}), which was 2–5 times higher than those observed for the other red pandas in the experiment. The P content remained relatively high from March 15 to May 25 and peaked on April 13 (2.08 ng mL^{-1}), and then dropped to 0.11 ng mL^{-1} on June 5 and remained at the base level.

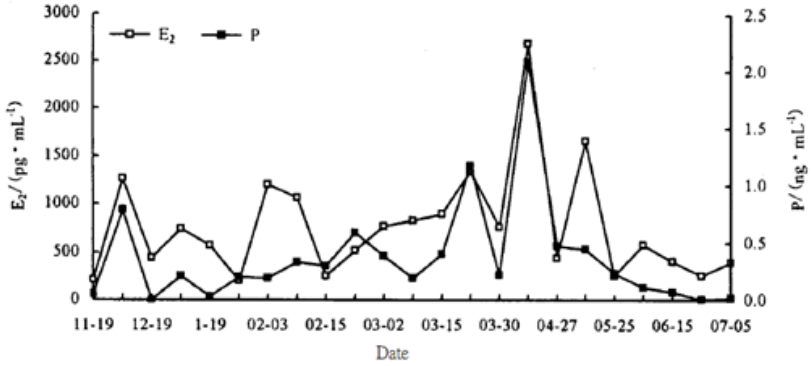

Figure-4. Serum concentration of estradiol and progesterone in animal 4.

Red panda 5 engaged in mating once each on February 21 and February 22 and gave birth to an infant red panda after 114 days. The changes in serum E_2 and P content are shown in Figure 5. The E_2 content peaked on February 21 (620.4 pg mL^{-1}). The E_2 content dropped rapidly following mating and remained below 360 pg mL^{-1} from March 2 to July 5. The P content remained low before the estrus period; it was 0.35 ng mL^{-1} on the day of mating and dropped subsequently. The P content increased markedly on March 9, peaked on April 13 (1.36 ng mL^{-1}), and remained at base level after May 25.

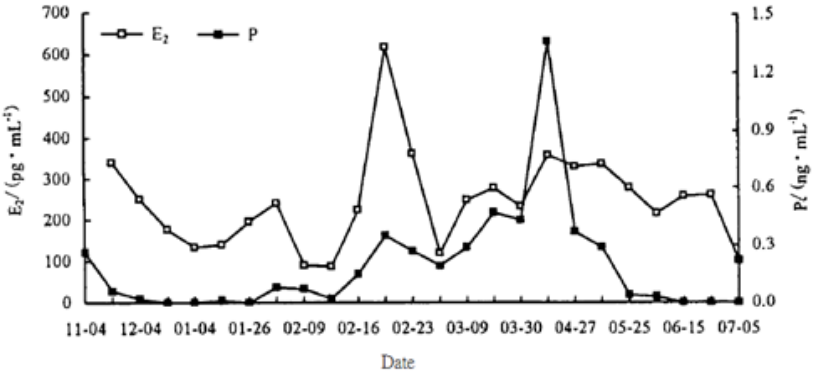

Figure-5. Serum concentration of estradiol and progesterone in animal 5.

4. DISCUSSION

E_2 is vital for inducing estrus periods in female mammals and constitutes the basis for the estrus period of female mammals. Several mammals demonstrate marked peaks in E_2 content prior to ovulation; at the peak of the estrus period in leopards, cheetahs, and domestic cats, the E_2 content in the feces of these animals increased several times [11-13]. The E_2 content observed in giant pandas before their estrus period remained at a relatively low level

but increased suddenly during the estrus period and peaked. After the estrus period, the E_2 content dropped rapidly to base level [14]. The experimental results of the present study revealed that the serum E_2 content in the red pandas remained at base level before the estrus period, peaked on the day of mating, and rapidly dropped to base level after mating. This finding is consistent with the findings of other studies [5, 7, 10] and confirms the importance of E_2 in inducing seasonal reproduction in red pandas. Changes in the E_2 content in female mammals during the estrus period can be used to determine whether such mammals have a single or multiple estrus periods. The results of the present study revealed that the E_2 content had several peaks during the red pandas' estrus periods. Red panda 1 had two peaks with an interval of 14 days, red panda 2 had two peaks with an interval of 25 days, red panda 3 had two peaks with an interval of 35 days, and red panda 4 had three peaks with intervals of 48 and 21 days. These findings are consistent with those of other studies [5, 7] and provide further support that the red panda is a seasonal animal with multiple estrus periods. On the basis of the observed intervals between the E_2 peaks, a relatively large variability exists in the estrus periods of red pandas. The periods ranged from 14 to 48 days, with the mean being 28.26 ±13.24 days (n = 5).

During the gestation period, the E_2 content of red pandas 1 and 2 observed after mating peaked after 34 days (March 15) and 52 days (April 13). One E_2 peak was observed for red panda 3, which was not pregnant, during the luteal phase (April 27). Moreover, two peaks were observed for red panda 4, which was not pregnant, during the luteal phase (April 13 and May 17). This finding is the first observation of an increase in the E_2 content in red pandas. On the basis of this finding, we can infer that ovarian follicle development may still occur in red pandas during the gestation period and luteal phase, and that the follicle can even reach high maturity. The same phenomenon is found in buffalo and cows. That is, following ovulation, another small follicle begins to develop; nevertheless, at this stage, the corpus luteum is not fully developed and P secretion is low. Endocrine disorders possibly stimulate follicle growth and increase the E_2 content. Due to the retardation of P, the secretion of this estrogen cannot stimulate hypophysis to release progesterone in abundance. This type of false estrus period does not involve ovulation, and because a normal amount of P maintains gestation, parturition may ultimately occur [15]. Further research is required to understand the reason underlying this phenomenon in red pandas. The P content in the red pandas increased during the gestation period of pregnant red pandas and the luteal phase of nonpregnant red pandas. In red pandas 1, 2, and 5, peak P levels were observed 34, 52, and 51 days after mating, respectively. In red pandas 3 and 4, peak P levels were observed 21 days after the most recent E_2 peak. The P content increases in preparation for gestation in every estrus period in mammals as the corpus luteum develops. P can maintain proliferation of the uterine mucous membrane and stimulate growth and increased bending of the uterine gland, forming uterine milk. Uterine milk is beneficial for nutrient absorption and embryo development and implantation in the early period. Moreover, P can inhibit uterine muscle autorhythmicity and maintains the uterus in a stable state [4]. The changes in P content in the pregnant and nonpregnant red pandas found in this study are consistent with the findings of Yunfang, et al. [10]. The P content increases and peaks after the estrus period in giant pandas, regardless of their pregnancy status [14]. Delayed implantation has evolved in numerous mammals to help them live and propagate effectively. This phenomenon of delayed implantation is often found in the family Ursidae Luan, et al. [16]. Tsubota, et al. [17] found that the serum P content in a pregnant Yezo brown bear (*Ursus arctos yezoensis*) increased slightly in the mating season (May to July). The serum P content also increased from September to October, and the rate of increase was intensified in the period from November to December, which is considered as a manifestation of delayed embryo implantation in Yezo brown bears. Embryo implantation is deduced to occur during the rapid increase in the P content. The serum P content in black bears (*Ursus americanus*) is extremely low in the early period of delayed implantation, and before implantation, the P content

increases substantially [18, 19]. Delayed implantation also occurs in giant pandas after conception; at this time, fertilized eggs are at the germinal vesicle stage and gradually enter the uterus, delaying the occurrence of the peak P content to the third month Hu [20]. Hu [1] reported that high-latitude and extremely cold weather conditions cause delayed implantation in red pandas to help them cope with the harsh environmental conditions. The red pandas then give birth under suitable environmental and climatic conditions. Roberts and Kessler [3] found relatively large changes in the gestation periods of red pandas (111 to 145 days). Accordingly, they deduced that embryos were not fully developed in the red pandas during the period and that their resting state engendered the changes. The present study determined that the serum P content started to increase in pregnant red pandas after mating. According to the change trend of the P content, we did not find evidence of delayed embryo implantation in captive breeding red pandas in Fuzhou.

5. CONCLUSION

(1) When female red pandas entered the estrus period, their serum E_2 levels increased rapidly and peaked several times. Only one peak value was consistent with mating behavior. This indicates that red pandas have a seasonal reproductive cycle with multiple estrus periods, with the average length of an estrus period being 28.26 ± 13.24 days (n = 5). (2) The E_2 content peaked during the gestation period of pregnant red pandas and luteal phase of nonpregnant red pandas. Accordingly, we can deduce that during the gestation period and luteal phase, follicle development still occurs in the ovary. (3) The serum P content increased during the gestation period of pregnant red pandas and luteal phase of nonpregnant red pandas. On the basis of the changes in the P content, no evidence of delayed embryo implantation was found in captive breeding red pandas in Fuzhou.

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