

# Testosterone Changes in Female Pandas in Estrus

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**Abstract:** Currently, the majority of giant panda breeding is carried out by cage-mating or artificial insemination based on estrogen levels and behavior in female pandas. However, studies have shown that testosterone levels both in women and in non-human primate females have a significant effect on the desire to mate. In this study, we wanted to explain how testosterone levels of female giant pandas would change during estrous. In this study, 23 accounts of rutting were recorded in 10 female pandas from 2009 to 2012. Changes in urinal testosterone levels were monitored and compared with estradiol values. Our data showed that, for female pandas in estrus, testosterone levels after the estradiol peak was significantly higher than before, and the testosterone peak occurred 4 days after the estradiol peak. Furthermore, testosterone and estradiol level were only significantly correlated after peak estradiol levels peaked, and not before. Finally,

23 out findings suggest that testosterone could help us better understand hormone variation during  
24 panda estrus, as well as help aid in the natural breeding of pandas.

25 **Key words:** Breeding; Fertility; Hormone variation; Rutting

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## 27 **1. Introduction**

28 Giant pandas (*Ailuropoda melanoleuca*) are rare in the world. Under artificial breeding  
29 conditions, a healthy female giant panda experiences one estrus period per year, from February to  
30 June. The duration is short and rutting behavior is usually observed. As estrus approaches, the  
31 vaginal orifice becomes red and swollen and the female may rub the genital region with a paw or  
32 on objects (Anon, 1974; Kleiman et al., 1979).

33 In recent years, many researchers studied changes in giant panda reproductive endocrine  
34 (Zeng et al., 1984; Shi et al., 1988; Peng et al., 1993; Xie et al., 1993) as well as variation in  
35 related hormones such as estradiol, progesterone, lutinizing hormone, etc. (Bonney et al., 1982;  
36 Hodges et al., 1984; Liu, 1988; Zeng et al., 1990; Li et al., 1993; Yu et al., 2003). These studies  
37 closely examined the relationship between hormones and ovulation. However, other studies found  
38 that testosterone levels in women and in non-human primate females also have a significant effect  
39 on the desire to mate (Everitt et al., 1971; Davis and Tran, 2001; Gao et al., 2007). Previous  
40 research has also shown that testosterone levels in women and sexual readiness showed a  
41 significant positive correlation (Sherwin et al., 1987; Van et al., 1997; Gumell and Chatterjee,  
42 2001). According to a study of Sichuan golden monkeys during their breeding period, solicitous  
43 behavior in three females showed significant positive correlation with their testosterone levels  
44 (Gao et al., 2007).

In our study, a radioactive immunity method was to monitor changes in testosterone during female giant panda breeding, and is compared with changes in estradiol. This research provides reference data for better understanding of the hormonal changes during the panda's estrus breeding period.

## 2 Materials and Methods

### 2.1 Ethics statement

The study was approved by the Beijing Municipal Committee of Animal Management before sample collection.

All experiments were performed in accordance with the approved guidelines and regulations.

### 2.2 Materials

Experimental animals included 10 female pandas from the rescue breeding research center in Shanxi and Beijing Zoo. From 2009 to 2012 urine was collected and examined a total of 23 times (Table 1). Urine was only measured after estrus behavior was observed. Estrus behavior in giant female pandas was defined by Bonney et al (1982). During the female estrus period, urine was collected between 8:00 to 10:00 A.M. every day and immediately stored in a - 20°C freezer. Day 0 is defined as the day of peak estradiol. One urine sample was collected daily from each animal starting 8 days before and 8 days after Day 0 for a total of 16 days.

**Table 1 Information of sampling**

Studbook	Name	Birth year	Sampling year	Institution
320	Lele	1986	2009, 2011	Beijing Zoo
403	Jini	1993	2010, 2011, 2012	Beijing Zoo
566	Yinghua	2003	2010, 2011, 2012	Beijing Zoo
652	Mengmeng	2006	2010, 2011	Beijing Zoo
444	Xuexue	1988	2009	The Rescue Breeding Research Center in Shanxi

509	Zhuzhu	2000	2009, 2011, 2012	The Rescue Breeding Research Center in Shanxi
562	Yangyang	2003	2009, 2011, 2012	The Rescue Breeding Research Center in Shanxi
603	Xinxin	2005	2011, 2012	The Rescue Breeding Research Center in Shanxi
660	Niuniu	1997	2009, 2011, 2012	The Rescue Breeding Research Center in Shanxi
699	Chengcheng	2006	2012	The Rescue Breeding Research Center in Shanxi

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## 65 2.3 Urine sample processing

66 Fresh urine samples are tested immediately. Cryopreserved urine was placed in ice baths and  
67 tested after it reached room temperature.

## 68 2.4 Hormone detection

69 Using the radioactiveimmunity method described by Monfort et al. (1989), with some slight  
70 modifications. The radiation immunoassay reagent kit used was from Beijing Kemeidongya  
71 Biological Technology Co. Ltd. and the GC2010 Ria Gamma Counting Instrument from Hefei  
72 ZhongCheng Co. were used for testosterone and estradiol measurement. Urine creatinine detection  
73 was performed with the creatinine (picric acid method) kits from Beijing Kemeidongya Biological  
74 Technology Co. Ltd. and the 7080 biochemical analyzer from Japan Hitachi.

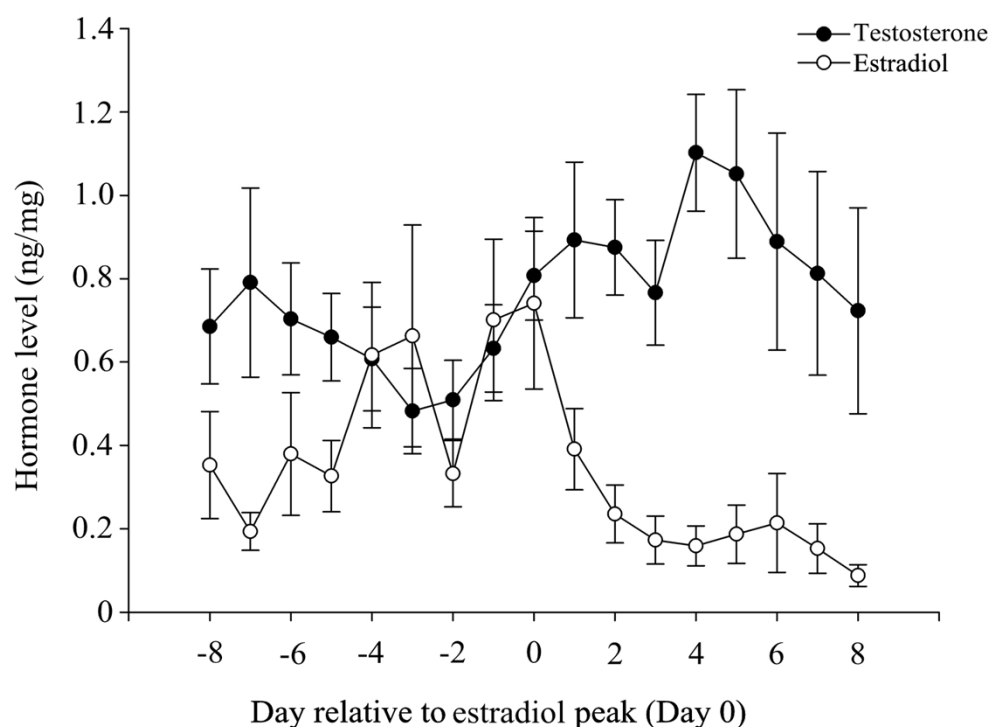
## 75 2.5 Data processing

76 Testosterone and estradiol measurement data were used for creatinine values for calibration.  
77 In order to eliminate possible errors, hormone concentrations with ria determination results and  
78 the ratio of creatinine content of the same sample. Statistical procedures were performed using  
79 SPSS 19.0.

## 80 3 Results

81 Statistical analysis was performed using the results of the 23 cases of rutting where the  
82 female giant pandas' testosterone and estradiol level (Mean  $\pm$  SE) and production curve were  
83 calculated (Fig. 1). Figure 1 shows that during the giant panda's estrus breeding period, in urine,

the testosterone levels peaked 4 days after estradiol levels peaked. For the spearman rank correlation test, testosterone and estradiol level were not significantly correlated before peak estradiol, while after that they were significantly correlated (Before:  $n = 118$ ,  $r = 0.124$ ,  $P = 0.182$ ; After:  $n = 99$ ,  $r = 0.239$ ,  $P = 0.017$ ). Furthermore, testosterone levels were significantly higher after estradiol peaked than before (Independent-Samples t Test:  $n = 250$ ,  $t = 1.975$ ,  $P = 0.044$ ).



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90 Fig.1 Levels of Urinary Testosterone and Estradiol of Female Giant Pandas during Estrus

## 91 4 Discussion and Conclusions

92 This was the first study to monitor variations in testosterone levels in female giant panda  
93 during estrus. We observed that its changes were similar to that of estradiol. Previous research in  
94 animal reproductive endocrinology showed that testosterone is a precursor of estradiol and  
95 testosterone can be converted into estrogen by aromatase. For pandas in estrus, urinary estradiol  
96 peaks earlier than testosterone by 4 days (Fig. 1). This may be due to the sudden increase of

97 ovarian secretion of testosterone or to the aromatase conversion function reduction. Whether or  
98 not this phenomenon is associated with ovulation is worthy of further research.

99 Some research indicate that optimal breeding time should be on the following day or even on  
100 the third day after the peak (Hodges et al., 1984; Zeng et al., 1984). Natural breeding of pandas  
101 also occurs after estradiol peaks (Peng et al., 1993). Furthermore, research has also shown that  
102 testosterone levels in females and sexual readiness showed a significant positive correlation  
103 (Sherwin et al., 1987; Van et al., 1997; Gumell and Chatterjee, 2001). According to a study of  
104 Sichuan golden monkeys during their breeding period, solicitous behavior in three females  
105 showed significant positive correlation with their testosterone levels (Gao et al., 2007). In this  
106 study, we found that testosterone levels are significantly higher after estradiol levels peak.  
107 Therefore, testosterone may be a new indicator to evaluate the natural breeding of pandas and  
108 provide a reference for further understanding hormone variation during panda fertility.

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