

# 1 Testosterone Changes in Female Pandas in 2 Estrus

3 Xuefeng Liu<sup>1</sup>, Lei Yuan<sup>1</sup>, Wei Wang<sup>1</sup>, Yuyan You<sup>1</sup>, Yanhui Liu<sup>1</sup>, Tao Ma<sup>1</sup>, Zezhong Wang<sup>1</sup>,

4 Xuelin Jin<sup>2</sup>, Qingyi Ma<sup>2</sup>, Yanping Lu<sup>1</sup>, Jinguo Zhang<sup>1,\*</sup> & Sufen Zhao<sup>1,\*</sup>

5 <sup>1</sup> Beijing Key Laboratory of Captive Wildlife Technologies, Beijing Zoo, Beijing, 100044, China.

6 <sup>2</sup> Shanxi Wild Animal Rescue and Conservation Research Center, Zhouzhi, Shanxi, 710402,  
7 China.

8 \* Corresponding author at: Beijing Key Laboratory of Captive Wildlife Technologies, Beijing Zoo,  
9 137 Xizhimen Outer St, Xicheng, Beijing, 100044, China,  
10 Telephone.: +86-10-68390483,

11 E-mail: [zhangjinguo001@yeah.net](mailto:zhangjinguo001@yeah.net), [zhaosufen111@126.com](mailto:zhaosufen111@126.com)

12 \* These authors contributed equally to this work.

13 **Abstract:** Currently, the majority of giant panda breeding is carried out by cage-mating or  
14 artificial insemination based on estrogen levels and behavior in female pandas. However, studies  
15 have shown that testosterone levels both in women and in non-human primate females have a  
16 significant effect on the desire to mate. In this study, we wanted to explain how testosterone levels  
17 of female giant pandas would change during estrous. In this study, 23 accounts of rutting were  
18 recorded in 10 female pandas from 2009 to 2012. Changes in urinal testosterone levels were  
19 monitored and compared with estradiol values. Our data showed that, for female pandas in estrus,  
20 testosterone levels after the estradiol peak was significantly higher than before, and the  
21 testosterone peak occurred 4 days after the estradiol peak. Furthermore, testosterone and estradiol  
22 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

26

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).

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

49

50 **2 Materials and Methods**

51 **2.1 Ethics statement**

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

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

55 **2.2 Materials**

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

63

**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

---

64

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.

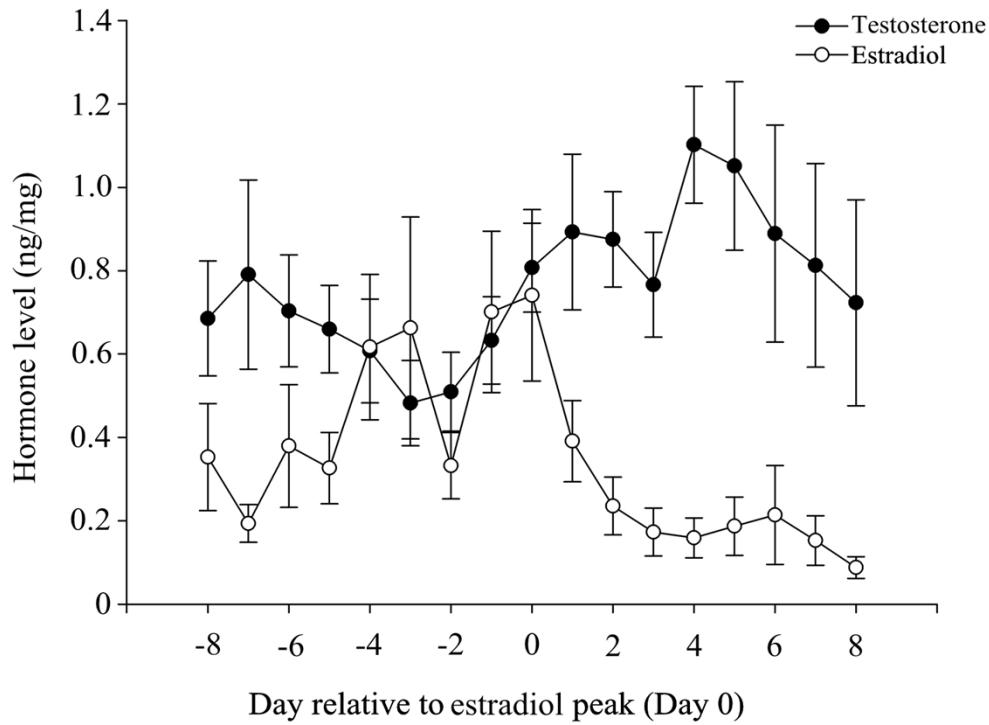
75 **2.5 Data processing**

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,

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



89  
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.

## 109 **Funding**

110 Beijing Zoo and Memphis Zoo Giant Panda Conservation and Research Projects.

## 111 **Acknowledgement**

112 This study was funded by the U.S. Memphis Zoo's Protection Project for Giant Pandas.  
113 Thanks to the Beijing Zoo and its staff in the Protection of the Rare Wildlife Rescue Breeding  
114 Research Center in Shanxi Province for providing research support.

## 115 **Reference**

116 Anon. 1974. On the breeding of the giant panda and the development of its cubs. *Acta zoologica*  
117 *sinica*. 20: 139 - 147.  
118 Baum M.J., A.K. Slob, F.H.D. Jong, and D.L. Westbroek. 1978. Persistence of sexual behavior in

119 ovariectomized stumptail macaques following dexamethasone treatment or adrenalectomy. Horm.  
120 Behav. 11: 323 - 347.

121 Bonney R.C., D.J. Wood, and D.G. Kleiman. 1982. Endocrine correlates of behavioural oestrus in  
122 the female giant panda (*Ailuropoda melanoleuca*) and associated hormonal changes in the male. J.  
123 Reprod. Fertil. 64:209 - 215.

124 Davis S.R. and J. Tran. 2001. Testosterone influences libido and well being in women. Trends  
125 Endocrinol . Metab. 12: 33 - 37.

126 Dixon A.F. 1990. The neuroendocrine regulation of sexual behavior in female primates. Annu.  
127 Rev. Sex Res. 1: 197 - 226.

128 Everitt B.J. and J. Herbert 1971. The effects of dexamethasone and androgens on sexual  
129 receptivity of female rhesus monkeys. J. Endocrinol, 51: 575 - 588.

130 Gao Y.F., H.P. Wang, and B.G. Li. 2007. Seasonal changes of fecal testosterone levels and  
131 solicitation frequency in female Sichuan snub-nosed monkeys (*Rhinopithecus roxellana*) the  
132 Qinling Mountains, ACTA Zoologica sinica. 53:783 - 790

133 Goldfoot D.A., S.J. Wiegand, and G. Scheffler. 1978. Continued copulation in ovariectomized  
134 adrenal2suppressed stumptail macaques (*Macaca arctoides*) . Horm. Behav. 11 : 89 - 99.

135 Gumell E.M., and V.K.K. Chatterjee. 2001. Mini-review: dehydroepiandrosterone replacement  
136 therapy. Eur. J. Endocrinol. 145: 103 - 106.

137 Hodges J.K., D.J. Bevan, M. Celma, J.P. Hearn, D.J. Jones, D.G. Kleiman, J.A. Knight, and  
138 H.D.M. Moore. 1984. Aspects of the reproductive endocrinology of the female giant panda  
139 (*Ailuropoda melanoleuca*) in captivity with special reference to the detection of ovulation and  
140 pregnancy. J. Zool. Lond. 203:253 - 267.

141 Li F.D., S.Y. Peng, Z.Y. Ye, G.G. He, and X.M. Huang. 1993. Female panda estrus urine 17 -  $\beta$   
142 estradiol and progesterone levels and the relationship with the breeding. ACTA Theriologica  
143 Sinica. 13: 166 - 171

144 Liu W.X. 1988. Research on Giant panda estrus in urine E2, LH Study on the Content.  
145 Research on Giant panda breeding and disease. Science and Technology Press, Sichuan.

146 Monfort SL, Dahl KD, Czekala NM, Stevens L, Bush M, Wildt DE. 1989. Monitoring ovarian  
147 function and pregnancy in the giant panda (*Ailuropoda melanoleuca*) by evaluating urinary  
148 bioactive FSH and steroid metabolites. J Reprod Fertil 85:203–12.

149 Peng S.Y., F.D. Li, Z.Y. Ye, X.M. Huang, and G.G. He. 1993. Female panda estrus and pregnancy  
150 urine hormone changes. Chinese Journal of Zoology. 28:25 - 28.

151 Shi S.Q., L. Dong, Y.C. Chen, and D.D. Feng. 1988. Research on Giant Panda estrus females of  
152 reproductive endocrine changes. ACTA Theriologica Sinica. 8 :1 - 6.

153 Sherwin B.B., and M.M. Gelfland. 1987. The role of androgens in the maintenance of sexual  
154 functioning in oophorectomized women. Psychosom. Med. 49:397 - 409.

155 Kleiman D.G., W.B. Karesh, and P.R. Chu. 1979. Behavioural changes associated with oestrus in  
156 the Giant panda (*Ailuropoda melanoleuca*) with comments on female proceptive behaviour.  
157 International Zoo Yearbook. 19:217 - 223.

158 Van G. S., V. Wiegant, E. Endert, F. Helmond, and P. N. Vande. 1997. Psychoendocrinological  
159 assessment of the menstrual cycle: The relationship between hormones, sexuality, and mood. Arch.  
160 Sexual Behav. 26:359 - 382.

161 Xie Z., W.X. Liu, and N.L. Liu. 1993. Giant Panda urine estradiol changes periodically preliminary  
162 study. Sichuan 93' Chengdu Giant Panda Protection International Symposium, Sichuan.

163 Yu S.R., Z.H. Zhang, J.Q. Yu, G.H. Li, A.J. Zhang, G.X. He, X.M. Huang, and R. Hou. 2003.

164 Research on the Changes of Sex Hormones in Female Giant Pandas Urine During Estrus and

165 Gestation. Chinese Journal of Animal and Veterinary Sciences. 34:452 - 456

166 Zeng G.Q., Z.B. Meng, G.T. Jiang, and G.X. He. 1984. Giant panda estrus females estrogen

167 content in urine and estrus behavior. ACTA Zoologica sinica. 30: 324 - 330.

168 Zeng G.Q., G.T. Jiang, K.Q. Yang, W.X. Liu, Z. Xie, and N.L. Liu. 1990. Panda estrus in serum

169 and urine luteinizing hormone, progesterone and  $17\beta$ -estradiol content changes. ACTA Zoologica

170 sinica. 31: 63 - 69.