SEASONAL CHANGES OF PROGESTERONE AND TESTOSTERONE CONTENTS IN HAIR OF GIANT PANDAS*

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ABSTRACT

Seasonal changes of progesterone content (PSC) in hair of three female giant pandas and testosterone content (TSC) in hair of two male giant pandas were tested. It was found that the two hormones could be detected by radioimmunoassay in all the hair samples scissored from the female and male giant pandas respectively. During March to early June, PSC in two non-pregnent giant pandas ($\bar{x} \pm SD = 13.40 \pm 10.06$ and 10.60 ± 8.88 ng / g hair respectively) were higher than those in non-breeding season (3.07 ± 1.07 and 3.20 ± 1.15 ng / g, $P < 0.01$). PSC in a 18-year-old female giant panda remained at low levels (2.72 ± 1.49 ng / g) during March to December. In a twin-bear giant panda, PSC (6.77 ± 3.66 ng / g) appeared higher than that in non-pregnant giant pandas in non-breeding season. Around February to the end of May, TSC in two male giant pandas (1.89 ± 1.71 and 1.82 ± 1.04 ng / g respectively) were also higher than that in non-breeding season (0.98 ± 0.57 and 0.75 ± 0.39 ng / g, $P < 0.01$). The findings from the study implied that giant panda's hair is possible to be used as a specimen to carry out steroids research in the endangered species.

Keywords Giant panda, Hair, Progesterone, Testosterone, Radioimmunoassay

1 INTRODUCTION

Giant panda (Ailuropoda melanoleuca) is one of the most precious natural heritages in the world. Based on some surveys, less than 1,100 remain in nature condition in China and most of them inhabit in Sichuan Province[1]. To improve the reproduction of this endangered species in captive and in the wild, intensive studies on its sex hormones are required. Some data on determination of progesterone

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concentrations in blood[2] or urine[3] of the giant pandas were showed. Urinary progesterone metabolites such as pregnanediol-3α-glucuronide (PdG) and 20α-hydroxypregn-4-en-3-one were evaluated connected with the observations on pregnancy status[4—6]. However, studies on progesterone and testosterone in giant panda are still limited, mainly because of the unavailability of the test animal, partly due to the problem in specimen collection. Frequent sampling of blood or milk in other species (e.g. dairy cow) is possible, but it is not the case in giant panda. Urine and faeces sometimes are easy to be contaminated by other substances when collected from the ground. In recent years, the authors found that progesterone could be detected in the hair in dairy cow[7], goat[8] and buffalo[9] and pregnency diagnosis could be made based on such determination. The present study was disigned to explore the possibility of detection of hair progesterone and testosterone in giant panda and to know the profiles of these hormones in different seasons.

2 MATERIALS AND METHODS

2.1 Animal

Three female and two male giant pandas raised at the Research and Conservation Centre for Giant Panda, Wolong Natural Reserve, were tested. The females included "Jia Jia", 11 years old (when the study began), with body weight 85kg; "Dong Dong", 6 years old, 89 kg; and "Li Li", 17 years old, 100 kg. The males included "Le Le", 6 years old, 107 kg; and "Zhen Zhen", 6 years old, 96 kg.

2.2 Hair sampling

For the females, 300 to 500 mg hair samples were taken with scissors from the back of the giant pandas at the intervals of 3 to 5 d during the breeding season (from the next half of Feb. to the end of May) and 7 d during the other seasons. For the males, hair samples were collected at 4 to 7 days intervals in the year. The hair specimens so collected were kept at 4—20°C for analysis.

2.3 Hormone assay

The hair segments were firstly extracted with petroleum ether. Then the progesterone was assayed by following the progesterone RIA Kit developed by Zoology Institute of Academia Sinica. The sensitivity of the assay was 12 pg/tube. The intra- and inter-assay precision was 8 % ($n=6$) and 13 % ($n=6$), respectively. The average recovery was 83 % ($n=6$). The relative activities of cross-reacting steroids in the assay were: progesterone 1.0; 17-hydroxyprogesterone 0.025; cortisone 0.0005; 11-deoxycortisol and cortisol 0.0001; aldosterone, oestrone, oestradiol and oestriol < 0.0001. The testosterone was assayed using the RIA Kit produced in Shanghai Endocrinology Institute.

During the experiment, physiological status, behavioural oestrus and other

reproductive activities of the giant pandas were observed.

3 RESULTS

3.1 Female giant panda

From the assays, it was found that progesterone could be detected in all the hair

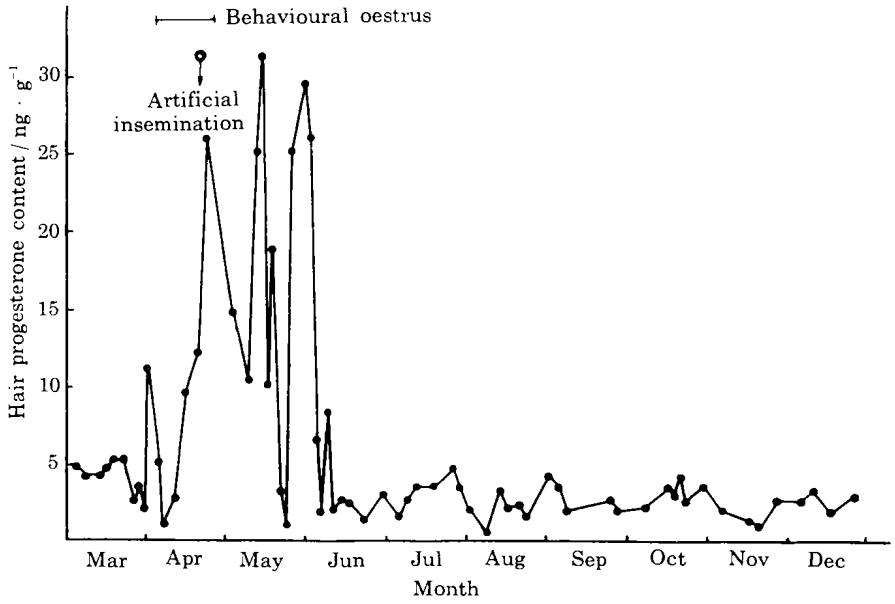

Fig.1 Hair progesterone profile in giant panda Jia Jia

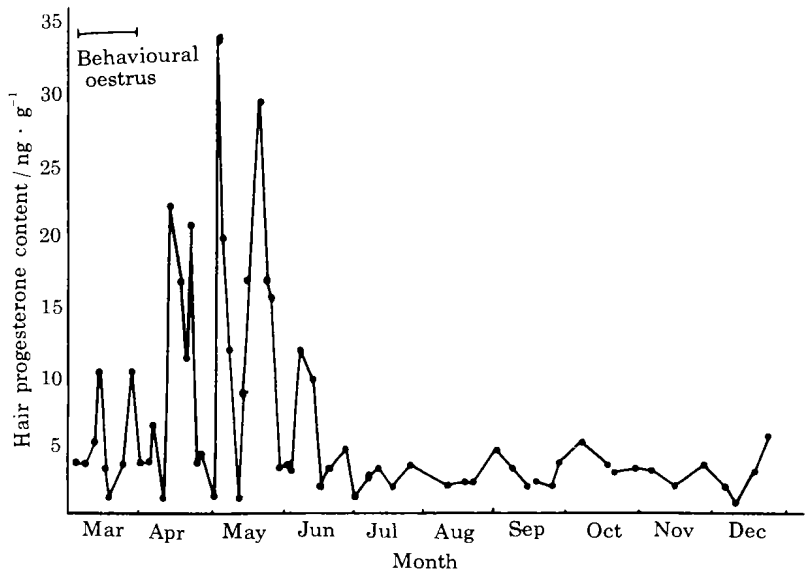

Fig.2 Hair progesterone profile in giant panda Li Li (1988)

samples collected from the female giant pandas in different seasons.

Jia Jia: from 11 to 29 April 1988, behavioural signs of oestrus such as increasing

anogenital rubbing, vocalizations and urinations, reduced appetite, etc. were showed intermittently. Artificial inseminations were made during 20 to 23 April. Hair progesterone contents in the measured period (4 March to 26 December) were 6.36 ± 7.45ng/g (mean ± SD) and during 1 April to 8 June were higher (13.40 ± 10.06 ng/g) than that in other seasons (3.07 ± 1.09 ng/g, $P<0.01$, Fig.1). No birth occurred.

Li Li: Rather weak oestrous signs showed disjointedly during the period of 2 March to 14 April 1988 (when 17 years old). No mating has been made. Hair progesterone contents in the measured period (from 4 March to 23 December) were 6.68 ± 6.95 ng/g and during 14 March to 15 June (10.60 ± 8.88 ng/g) were higher than that in other seasons (3.20 ± 1.15 ng/g $P<0.01$, Fig.2).

In 1989, no significant behavioural sign of oestrus in Li Li was observed and its hair progesterone kept at low levels all the year (2.72 ± 1.49 ng/g).

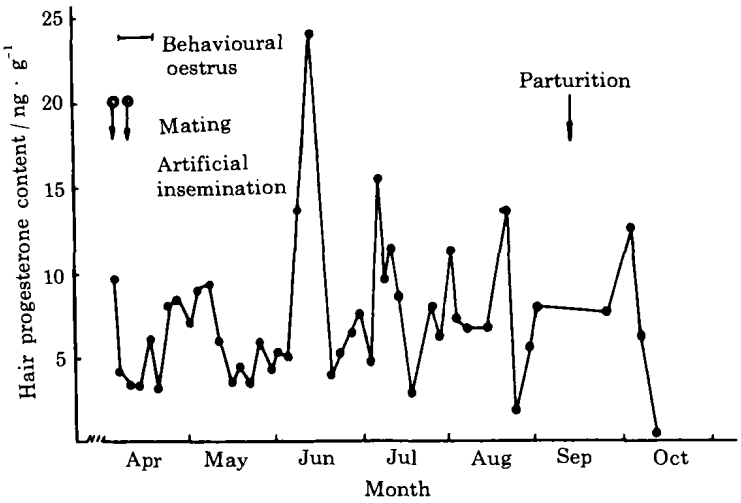

Fig.3 Hair progesterone profile in giant panda Dong Dong

Dong Dong: this panda was rescued from the wild in Feb. 1991 and has been reared in Wolong Reserve since 3 March 1991. It showed oestrus during 1 to 12 April and mated with male panda "Pan Pan" on 6, 7 and 8 April. Additional inseminations were made on 10 and 11 April. A twin was born on 7 September. No significant hair-progesterone rise was observed, but its mean value ($\bar{x}$ ± SD = 6.77 ± 3.66 ng/g) appeared higher than that of the Jia Jia and Li Li in non-breeding seasons (Fig.3).

3.2 Male giant panda

The experiments revealed that testosterone could be measured in all the hair specimens collected from the male pandas.

Le Le: During the next half of January to the end of April 1990, Le Le showed some signs of sexual urge such as frequent cry, urination and movement, fond of female panda, sticking out of the penis etc. His hair testosterone contents in 18

January to 19 May (1.82 ± 1.04 ng/g) was higher than that in other seasons (0.75 ± 0.39 ng/g, $P < 0.01$ Fig.4). In the overall experiment period (from 18 January to 26 December), the hair testosterone was at the level of 1.21 ± 0.91 ng/g.

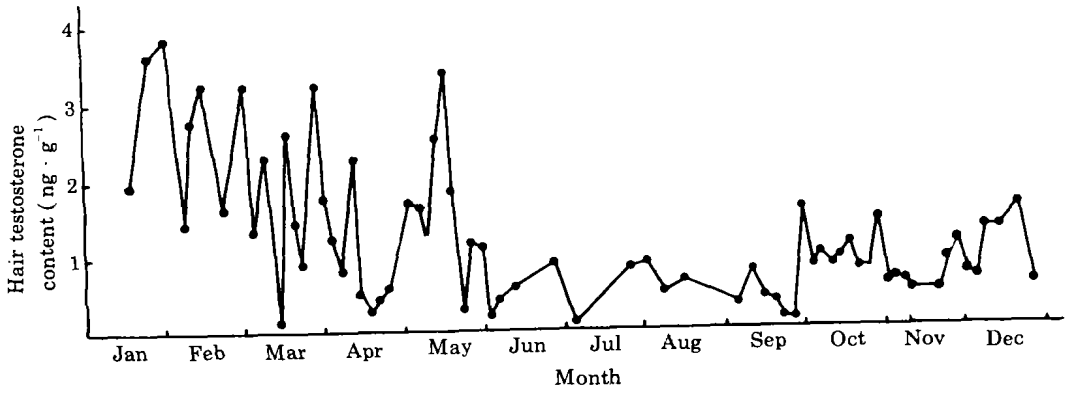

Fig.4 Hair testosterone profile in giant panda Le Le

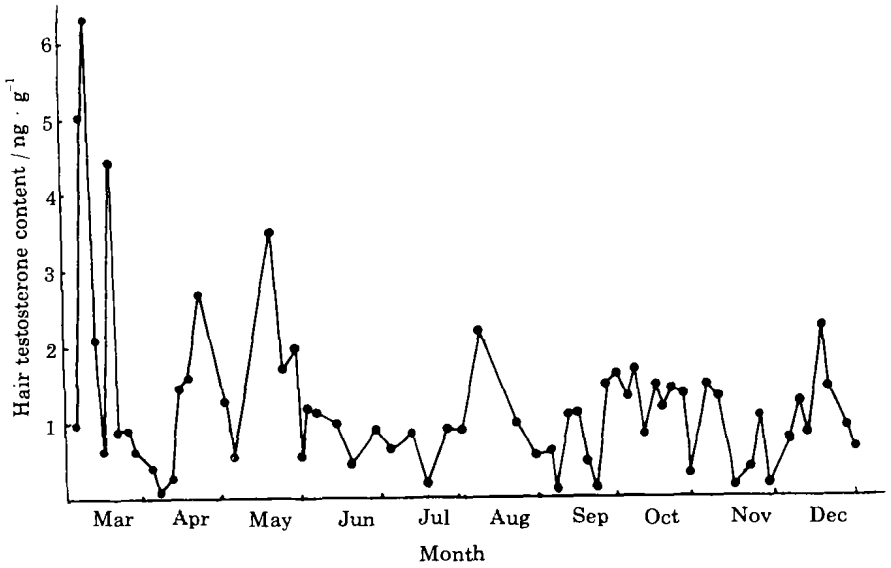

Fig.5 Hair testosterone profile in giant panda Zhen Zhen

Zhen Zhen: signs of sexual urge showed in March to May 1990. Its hair testosterone contents in the experiment period (from 5 March to 29 December) were 1.27 ± 1.12 ng/g, and during 5 March to 28 May (1.89 ± 1.71 ng/g) were higher than that in other seasons (0.98 ± 0.57 ng/g, $P<0.01$, Fig.5).

4 DISCUSSION

The results obtained from the study showed that progesterone and testosterone could be measured by radioimmunoassay in female and male giant pandas' hair respectively. The timing of the rise in hair progesterone contents in two female giant

pandas (Jia Jia and Li Li, 1988) and hair testosterone contents in two male giant pandas (Le Le and Zhen Zhen) coincide with the breeding season, which appeared reflecting the existed physiological process in these animals.

There was no significant hair-progesterone rise in Dong Dong during gestation, its implication remained to be elucidated, but showed a relatively higher levels compared to that of the non-pregnant Jia Jia and Li Li during non-breeding season. When monitoring the urinary concentrations of pregnanediol-3α-glucuronide in a pregnant giant panda, Chaudhuri *et al.*[5] also found that this progesterone metabolite increased from <5 to 15—30 ng/mg Cr after presumed ovulation, but increased further to 60—80 ng/mg Cr during 1 to 4 weeks before parturition. Monfort *et al.*[6] found that concentrations of pregnanediol-3α-glucuronide in urine increased by week 9 of gestation, and 20α-hydroxypregn-4-en-3-one levels increased 10—30 fold between 11th to 14th weeks. Presumably, different physiological process existed in the special monoestrous species and in different individuals. It seems that determination of progesterone or that of its metabolites alone will not suffice for pregnant diagnosis in giant panda.

Based on the observations of the hair-progesterone profile and behavioural sign of oestrus of Li Li in 1988 and 1989, one can suggest that the animal must lost reproductive capacity from 1989 when she was 18 years old.

The findings got from the present preliminary study implied that the giant panda's hair also can be used as specimen to carry out steroids research. If this approach could be verified in the following studies, more meaningful information could be gathered from the steroids research by using such method in the precious species since hair sampling has the following advantages: it is harmless to the panda; frequent sampling is possible since a small amount of hair (100—500 mg) is enough for one sampling; hair can be taken in any season and in most of the physiological status of the animal.

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