Plasma concentration of progesterone and 17β-estradiol of black-rumped agouti (*Dasyprocta prymnolopha*) during the estrous cycle

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Abstract: Plasma concentration of progesterone and 17β-estradiol of black-rumped agouti (Dasyprocta prymnolopha) during the estrous cycle. The agouti is a game animal that have been raised in captivity for conservation and sustainability purposes. However, the management of wild animals in an intensive breeding system requires an assertive knowledge of its reproductive parameters, one of the most important features for production improvement. Besides, little information is available regarding changes in reproductive hormone profiles in agouti. The objective of this study was to evaluate the hormonal profile of progesterone and 17β-estradiol during the estrous cycle of the agouti (*Dasyprocta prymnolopha*). The hormones were analyzed by radioimmunoassay. Blood samples were collected without sedation twice a week. The concentrations of progesterone were as follows: proestrus 0.78±0.39ng/ml, estrus 2.83±2.34ng/ml, metestrus 1.49±1.24ng/ml, diestrus 3.71±1.48ng/ml. In the estrous phase, an increase in the progesterone level was observed during a period of 24h. The average 17 β-estradiol levels were as follows: proestrus 2 030.98±961.00pg/ml, estrus 1 910.56±650.54pg/ml, metestrus 1 724.83±767.28pg/ml, diestrus 1 939.94±725.29pg/ml. The current results suggest that the progesterone plasma concentration during the estrous cycle in the agouti has a similar increasing, stabilizing and decreasing pattern, as in domestic mammals. Agoutis have two phases of follicular development, as two periods of 17β-estradiol peaks were observed, the first one in the metestrus and the second during the proestrus. Spontaneous ovulation seems to occur after the progesterone peak, possibly indicating that this hormone is associated with the ovulatory process. A more detailed investigation is needed for better understanding of how progesterone influences ovulation. Studies on the involvement of progesterone in follicular rupture can be carried out, using steroid biosynthesis inhibitors and observing the effect of this hormone on ovarian activity of proteolytic enzymes in the follicular wall. Rev. Biol. Trop. 59 (1): 29-35. Epub 2011 March 01.

Key words: *Dasyprocta prymnolopha*, reproduction, estrous cycle, progesterone, 17β-estradiol, Brazilian Amazon.

Successful wildlife management is directly related to reproduction programs, which require hormone monitoring to determine the estrous cycle, onset of puberty, duration of sexual receptivity, effect of season, pregnancy diagnosis, birth timing and physical fitness (Wildt & Wemmer 1999). Reproductive hormones, which can be measured in urine, feces, saliva and blood, are fundamental to regulate reproductive functions and may be used to improve reproductive management.

The reproductive endocrinology of most wild mammals is largely unknown and the endocrine physiology of Amazon animals is even less

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known. Understanding how the animals reproduce may contribute to improve reproduction biotechnologies, including artificial insemination, *in vitro* fertilization, embryo transfer and cryopreservation (Fickel *et al*. 2007). These are very important tools for *in situ* and *ex situ* wildlife conservation and animal production.

Native of Central and South America animals, such as the rodents, paca *Agouti paca*, capybara *Hydrochoerus hydrochaeris* and agouti *Dasyprocta* sp.*,* have potential for increase microlivestock production, since they represent the main protein sources for local populations (Bonaudo *et al*. 2005, National Research Council 1991).

Considerable effort has improved the knowledge of the reproductive physiology of the agouti (Guimarães 1993, 2000, Guimarães *et al*. 2009, Heap *et al*. 1981, Miglino *et al*. 2002, 2004, 2008, Rodrigues *et al*. 2006, Weir 1971, 1974); however, this species still requires further study.

The agouti is one of the most consumed game animals in the Brazilian Amazon (Bonaudo *et al*. 2005). An intensive breeding system of this animal will contribute to its conservation and can provide an alternative sustainable production.

Little information is available regarding changes in progesterone and estradiol levels during estrous cycle in agouti, compared to that in other hystricomorphs rodents (Adjanohoun 1992, Barta & Jakubicka 1991, Croix & Franchimont 1975, Faulkes *et al*. 1990, Montes & Baz 2006, Rowlands *et al*. 1970, Tam 1973, Van Aarde 1985). It is expected that the physiological parameters of progesterone and estrogen during the estrous cycle in agouti are similar to other hystricomorphs.

The aim of this work was to study the estrous cycle of the agouti (*Dasyprocta prymnolopha*) by analysis of reproductive hormones, progesterone and 17β-estradiol.

MATERIAL AND METHODS

This research was conducted at the wildlife paddock (4m^2) of the Animal Reproduction Laboratory of the Federal University of Pará, Belém, Brazil. Agoutis (*Dasyprocta prymnolopha*) were kept under natural lighting conditions. The paddocks annual average temperature was 28°C. Daily food supply was composed of corn bran (*Zea mays*), soy (*Glycine hispida*), pumpkin (*Curcibita pepo*), cassava (*Manihot utilissima*) and mineral salt; a nutritional complement (Potenay- Fort Dodge, Brazil) was provided periodically. All animals were born in captivity and belonged to F3 and F4 generations. The average weight of the experimental animals was 1.95±0.31kg.

The animals were divided into two groups, young and adult females. To compare the level of progesterone of nulliparous and pluriparous females and also to clarify whether ovulation occurs spontaneously or is copulation-induced, 18 females and 7 males were selected. The experimental groups were as follows:

Young females (9 months old): four groups of two nulliparous females and one vasectomized male.

Adult females (3 to 5 years old): three groups of two pluriparous females and one vasectomized male, and two groups of two pluriparous females kept isolated without a male.

Blood samples were collected without sedation twice a week (72h and 96h intervals) or in shorter periods (6h to 48h) next to the estrus by saphenous venipuncture. A 1ml syringe containing sodium heparin (Liquemine - Roche, Brazil) with a 26 ½ gauge needle (Baas *et al*. 1976) was used.

After collection, the blood was put in polypropylene tubes and immediately centrifuged at 1 000rpm for 10min. The plasma was stored at -20ºC until assaying. Progesterone concentrations in plasma were determined for all females and 17β-estradiol was measured in five of the adult coupled females.

The hormone levels were measured by radioimmunoassay I^{125} in the solid phase (Diagnostic Products Corporation, USA, for dosage of progesterone; Immunotech, France, for dosage of 17β estradiol). The assay quality control samples containing high and low hormone concentrations were included in the beginning

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and at the end of each assay. Intra-assay and inter-assay coefficient of variations were 11% and 10%, respectively. The progesterone assay specificity was 100% (17-hydroxyprogesterone 0.3%, 20 α-dihydroprogesterone 2.0%) and sensitivity was 0.02ng/ml. The 17β estradiol assay specificity was 100% (estrone 1.3% and estriol 0.65%) and sensitivity was 3pg/ml.

Colpocytology was performed to confirm the phase of the cycle and was applied according to Guimarães (1993), were as follows: proestrus-higher frequency of superficial and intermediate cells; estrus- higher number of superficial cells; metestrus-predominance of intermediate cells and leukocytes; diestrus-prevalence of parabasal cells and the increase of basal cells.

Statistical analyses were performed using BioEstat version 5 (Ayres *et al*. 2008). The level of significance throughout the study was 0.05 ($p<0.05$). Statistical differences on hormonal levels for each phase of the cycle were estimated by analysis of variance (ANOVA). Values are expressed as the mean ± standard deviation.

RESULTS

Twenty estrous cycles (32.05±4.17 days, 25 to 40 days) were verified in the adult females (n=10) and no statistical difference ($p>0.05$) was observed in cycle length between mated and isolated females. The high levels of progesterone observed in isolated adult females after estrus confirmed spontaneous ovulation.

Comparing young and adult females, there was no statistical difference ($p> 0.05$) in the progesterone profile for each phase of the estrous cycle. However, the average progesterone concentration among the different phases of the estrous cycle phases was statistically significantly different ($p<0.05$) (Table 1).

In the estrus, the initial progesterone concentration was low but it quickly increased in less than 24h and seemed to remain constant for more than 31h. The progesterone concentration decreased in approximately 6h (Table 2). These data suggest that the estrus length is approximately 24h and that ovulation happens after the progesterone concentration peak.

There was no statistical difference ($p>0.05$) in the 17β-estradiol levels between the cycle phases (Table 3). However, two peaks periods of 17β-estradiol were observed for all samples, the first in the metestrus and the second during the proestrus (Fig. 1), suggesting that there are two phases of follicular development.

The 17β-estradiol concentrations of one female, proestrus 77.26±20.71pg/ml (62.62-91.91pg/ml), estrus 82.24±10.96pg/ml (74.49-90pg/ml), metestrus 75.88pg/ml, diestrus 85.74±13.59pg/ml (71.93-109.37pg/ml), were low when compared to the samples mentioned above (Fig. 2).

DISCUSSION

The current results suggest that plasma progesterone concentration during the estrous cycle in the agouti (*D. prymnolopha*) define an increasing, stabilizing and decreasing pattern similar to those of domestic mammals. We have observed that the progesterone values reported here were similar to many hystricomorph rodents, as reported previously for the

TABLE 1

*Plasma level of progesterone (ng/ml) during estrous cycle of 18 agoutis (*D. prymnolopha*)*

Phase	Mean	Standard Deviation	Minimum	Maximum	Total samples
Proestrus	0.78	±0.39[a]	0.22	2.04	90
Estrus	2.83	±2.34[b]	0.34	9.02	31
Metestrus	1.49	±1.24[c]	0.23	5.64	60
Diestrus	3.71	±1.48[d]	0.91	8.38	267

a,b,c,d   Values with letters different in the same column are statistically significant ($p<0.05$).

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TABLE 2

*Plasma level of progesterone (ng/ml) during the estrus of adult agoutis (*D. prymnolopha*)*

P4 (ng/ml) - 1st harvest	P4 (ng/ml) - 2nd harvest	Time (hours)- between 1st and 2nd sample	Hormone levels
1.72	3.05	6	Increase
1.71	13.39	24	Increase
2.02	3.21	24	Increase
4.38	5.41	24	Increase
8.46	7.94	31	Decrease
8.33	1.63	6	Decrease
8.01	1.04	24	Decrease
7.87	5.92	24	Decrease
6.23	1.42	6	Decrease
4.7	1.08	6	Decrease
3.99	2.06	24	Decrease

TABLE 3

*Plasma level of 17☐estradiol (pg/ml) during estrous cycle of four agoutis (*D. prymnolopha*)*

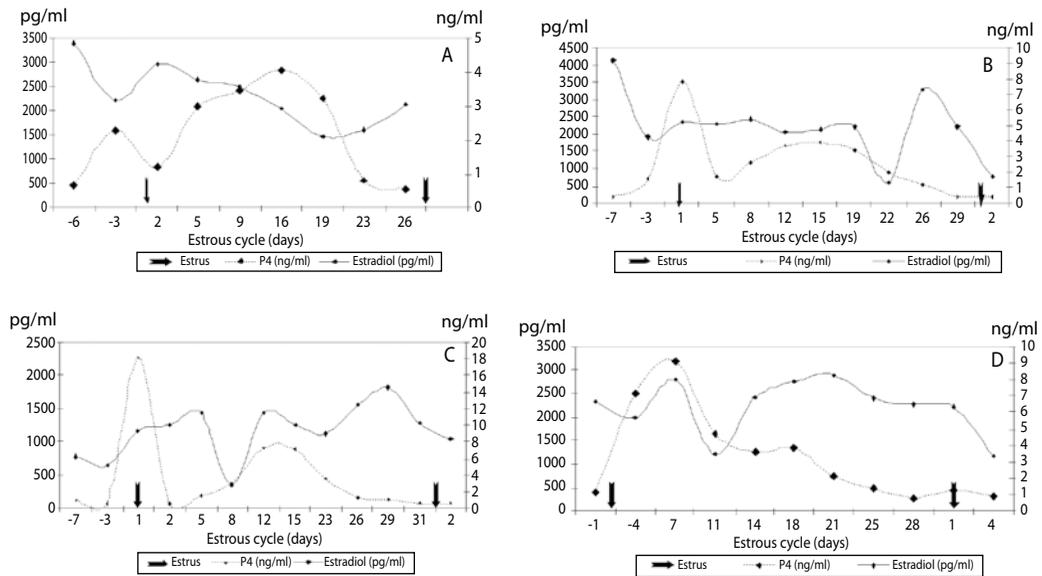| Phase	Mean	Standard Deviation	Minimum	Maximum	Total samples
Proestrus	2 030.98	±961.00	598.28	4 137.53	17
Estrus	1 910.56	±650.54	1 162.91	2 347.43	3
Metestrus	1 724.83	±767.28	765.64	2 956.58	9
Diestrus	1 939.94	±725.29	357.6	2 897.75	16

Fig. 1. Plasma estradiol-17β (pg/ml) and progesterone (ng/ml) levels during the estrous cycle of four agouti females (A, B, C, D).

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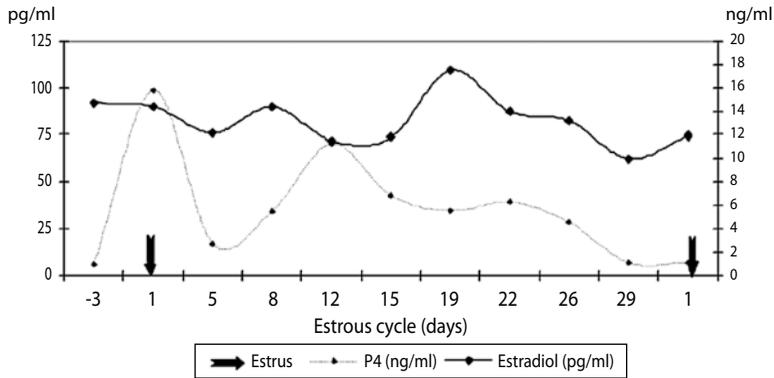Fig. 2. Hormonal profile of estradiol-17β (pg/ml) and progesterone (ng/ml) during the estrous cycle of one agouti (*D. prymnolopha*).

Cavia porcellus (Croix & Franchimont 1975), *Hystrix africaeaustralis* (Van Aarde 1985) and *Agouti paca* (Montes & Baz 2006). However, these values were lower than those observed in *Myoprocta pratti* (Rowlands *et al*. 1970), *Galea musteloides* (Tam 1973) and *Hetreocephalus glaber* (Faulkes *et al*. 1990). On the other hand, the data reported here were high, when compared to those observed in *Myocastor coypus* (Barta & Jakubicka 1991) and *Thryonomys swinderianus* (Adjanohoun 1992). Presumptive the intra-species variation in progesterone levels is based on the function of the *corpus luteum* correlated with the metabolism of this steroid hormone.

Spontaneous ovulation occurred after the progesterone peak, possibly indicating that it is associated with the ovulation process. This peak of progesterone was similar to that described by Feder *et al*. (1968) and Croix & Franchmont (1975) for the *Cavia porcellus*. In this species, the peak occurred a few hours before ovulation, which suggests the importance of this hormone in the ovulation process in some hystricomorph rodents. The pre-ovulatory follicles are probably responsible for a high production of progesterone during estrus. This may be one of the most important aspects of this study, however future research should be performed to confirm.

We found that the levels of 17β-estradiol during the estrous cycle showed an individual variation, as was also reported for the *Cavia porcellus* (Croix & Franchimont 1975) and *Hystrix africaeaustralis* (Van Aarde 1985). In effect, we believe that the agoutis appear to have two phases of follicular development, since two periods of 17β-estradiol increase were found. This information will be useful when implementing reproductive biotechniques for conservation of this species.

In addition to these characteristics that have been outlined, reduced levels of 17β-estradiol were observed only in one female. Probably, the occurrence of low levels of this hormone may be correlated with slow follicular development. This has also been observed previously in species such as the *Cavia porcellus* (Croix & Franchimont 1975), *Hystrix africaeaustralis* (Van Aarde 1985) and *Agouti paca* (Montes & Baz 2006).

This study is the first attempt to interpret the physiological events during the estrous cycle of the agouti. The present results indicate that plasma steroids (progesterone and 17β-estradiol) monitoring provides an effective tool for obtaining information to improve reproductive programs of agouti conservation and production. Moreover, the data reported here also suggest the need for a more detailed investigation to better understand how progesterone influences the ovulatory mechanism in the agouti.

ACKNOWLEDGMENTS

RESUMEN

El conocimiento de los procesos reproductivos de especies de importancia económica local son indispensables para apoyar su producción en cautiverio y garantizar su manejo sostenible. El objetivo del presente trabajo fue evaluar los niveles hormonales de progesterona y 17β-estradiol durante el ciclo estral en agutí (*Dasyprocta prymnolopha*). La recolección de sangre se realizó dos veces por semana, sin sedación. Las hormonas fueron analizadas por radioinmunoanálisis. Los niveles de progesterona fueron los siguientes: proestro 0.78 ± 0.39ng/ml, estro 2.83 ± 2.34ng/ml, metaestro 1.49 ± 1.24ng/ml y diestro 3.71 ± 1.48ng/ml. En el estro se observó un aumento de los niveles de progesterona durante un periodo de 24h. Los niveles de 17 β-estradiol fueron los siguientes: proestro 2 030.98 ± 961.00pg/ml, estro 1 910.56 ± 650.54pg/ml, metaesto 1 724.83 ± 767.28pg/ml y diestro 1 939.94 ± 725.29pg/ml. Los resultados encontrados sugieren que los niveles plasmáticos de progesterona durante el ciclo estral en agutí siguen un patrón de aumento, estabilización y diminución, tal como en los mamíferos domésticos. Agutí tienen dos etapas de desarrollo folicular, puesto que se observaron dos altos valores de 17β-estradiol, el primero en el metaestro y el segundo durante el proestro. La ovulación espontánea ocurre posiblemente después del aumento de la progesterona, indicando que esta hormona posiblemente está asociada con el proceso ovulatorio. Es necesario desarrollar un estudio más detallado para mejorar la comprensión del papel de la progesterona en la ovulación. Algunos estudios de la participación de la progesterona en la ruptura folicular se pueden realizar utilizando inhibidores de la biosíntesis de esteroides y observar el efecto de esta hormona sobre la actividad de las enzimas proteolíticas en la pared folicular.

Palabras claves: *Dasyprocta prymnolopha*, reproducción, ciclo estral, progesterona, 17β-estradiol, Amazonia brasileña.

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