REVISTA DE Biología Tropical

Profile of plasmatic progesterone on pregnancy, and the postpartum estrus of *Dasyprocta prymnolopha* (Rodentia: Dasyproctidae)

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Received 23-IX-2015. Corrected 06-V-2016. Accepted 07-VI-2016.

Abstract: The agouti (*Dasyprocta* sp.) is a hystricomorph rodent found in some regions of the Americas. It is an important cynegetic species, which indicates that the overhunting is a threat to their conservation. Very little is known about this wildlife in relation to what already has been studied in domestic animals. Thus, the knowledge on reproduction of wildlife becomes necessary and essential for the management and conservation of these natural resources. Specifically, studies regarding hormonal monitoring are important as a basic tool for research in modern reproductive biotechnology, and currently, there is no information on the progesterone changes during pregnancy of *Dasyprocta* sp., compared to other hystricomorphs. The aim of this study was to describe the profile of plasmatic progesterone during pregnancy, and report the restart of ovarian cycle in agouti after parturition. For this purpose, 18 black-rumped agoutis (*Dasyprocta prymnolopha*) born in captivity were used, and one or more consecutive pregnancies were observed. Copulation was confirmed by the presence of spermatozoa observed in the colpocytological examination. Blood samples were collected two times per week, and concentrations of progesterone, assessed in ten agoutis, were determined by radioimmunoassay. The onset of ovarian activity in six females was observed daily by colpocytological examination, starting on the seventh day postpartum. The gestational period observed in this study was 104.04 days (SD = 1.31) (101-106 days) (Number of cases, N = 26), and the interval between births was 126.03 days (SD = 18.40) (109-184 days). The plasmatic profile of progesterone during pregnancy showed a progressive increase from the 1st to the 5th week. The higher progesterone levels over this period (6.88 ng / mL, SD = 3.01) were detected in the 5th week. This value was similar (One-Way ANOVA, $p > 0.05$) to that observed in the 4th and 6th weeks, but was statistically different (One-Way ANOVA, $p < 0.05$) when compared to the other weeks. After the 6th week there was a progressive decrease in plasmatic progesterone levels. The animals showed a postpartum estrus of 12.04 days (SD= 4.29) (7-24 days) (N= 23). It was observed that 80.95 % (N= 19) of copulations during this period were fertile. This work contributed to understanding the dynamic changes in the progesterone levels during the pregnancy in agouti. Nevertheless, more studies are needed for a better appreciation of other endocrine and biological changes, in the mother and feto-placental unit of the agouti. Rev. Biol. Trop. 64 (4): 1519-1526. Epub 2016 December 01.

Key words: hystricomorph, agouti, gestation, postpartum, progesterone.

The hystricomorph rodents, such as paca (*Cuniculus paca*), agouti (*Dasyprocta* sp.) and other wild mammals, serve as animal protein sources for local rural communities (Valsecchi, El Bizri, & Figueira, 2014; Van Vliet et al., 2015), which usually leads to overhunting in the Amazon. This socio-environmental impact in this region, makes the management of wildlife an essential tool for food sovereignty and biodiversity conservation (Bonaudo, Le Pendu, Faure, & Quanz, 2005).

However, to achieve success in management of wild mammals, the study of its reproductive biology is required (Andrabi &

Maxwell, 2007), since it is poorly understood (Wildt & Wemmer, 1999; Fickel, Wagcher, & Ludwig, 2007). Knowledge of reproductive physiology, through endocrine monitoring is a key tool for animal husbandry practices, as well as integrated studies in different areas, such as ethology and ecology (Pereira, 2007). Few reproductive techniques have been used for the management of wild animals, including hormonal monitoring. Thus, all the technology has value to generate new information for species which are poorly studied. When there is availability of basic data, such as knowledge of reproductive hormones, it will be possible to use modern biotechnology techniques (i.e. artificial insemination, embryo transfer, cryopreservation of gametes and intracytoplasmic sperm injections, for genetic management and conservation of the species (Pukazhenthi, Comizzoli, Travis, & Wildt, 2006).

Long gestation periods are observed in tropical mammals. Some authors suggest that is due to adaptations to the environment more favorable to births, within a longer period of time (Kiltie, 1982), and others to the precocity of their young (Martin & MacLarnon, 1985). In the suborder hystricomorph, this aspect would be associated with slow fetal growth, especially in the early embryological stages (Roberts, 1971). The gestation period in agouti is about three and a half months, giving birth to two precocial young (Brown, 1936; Roth-Kolar, 1957; Nieuwendijk, 1980; Sousa et al., 2012; Fortes et al., 2013). The *corpus luteum* synthesizes progesterone during the first weeks of pregnancy in hystricomorphs and then the placenta performs this function (Tam, 1970, 1974). In this suborder, the beginning of pregnancy is characterized by low progesterone levels compared to more advanced stages. After this period, there is a progressive increase in the hormone levels, which gradually decrease near parturition (Tam, 1974).

In the literature, there is no information on the progesterone changes during the pregnancy of *Dasyprocta* sp., compared to other hystricomorph, such as *Myoprocta pratti*, *Cavia porcellus*, *Galea musteloides*, *Dolichotis patagonum*, *Myocastor coypus*, *Erethizon dorsatum*, *Hystrix africaeaustralis*, and *Cuniculus paca* (Rowlands, Tam, & Kleiman, 1970; Challis, Heap, & Illingworth, 1971; Tam, 1973; Louis, Somes, Pryor, & Blankenship, 1986; Van Aarde & Potgieter, 1986; Jakubička, Barta, Nitray, & Szeleszczuková, 1989; Barta & Jakubička, 1991; Sweitzer & Holcombre, 1993; Van Wyk, Van Aarde, & Louw, 1994; Ribeiro et al., 2012). Thus, the aim of this study was to describe the changes in the levels of progesterone during pregnancy, and report the restart of ovarian cyclicity postpartum in agoutis.

MATERIAL AND METHODS

The black-rumped agoutis (*Dasyprocta prymnolopha*) were born in captivity, at a wildlife paddock of the Federal University of Pará, Belém, Brazil (1º27' 21" S - 48º30' 14" W). Each female was housed with a male in breeding paddocks of 4 m^2, with a concrete floor and roof, and under natural lighting and ambient temperature (28 ºC) conditions. Daily food supply was composed of cob corn (*Zea mays*), soybean (*Glycine hispida*), pumpkin (*Curcibita pepo*), cassava (*Manihot utilissima*), mineral salt, water *ad libitum*, with oral administration of a nutritional complement two times a week (Potenay Oral NF - Fort Dodge Saúde Animal LTDA, Brazil), using a veterinary oral dosing syringe (M68 syringes for vermifugation - Aparelhos veterinários Höppner LTDA, Brazil). The protocol of this experiment was carried out in accordance with the Guide for the care and use of laboratory animals (National Research Council, 2011).

A total of 18 adult females of agouti aged more than 24 months, and weighing 2 kg on average, were selected. Each female was kept in their respective breeding paddocks (4 m^2). One or more consecutive pregnancies were observed in each female. Copulation was confirmed by the presence of spermatozoa in the colpocytological examination, and day zero corresponded to the day of mating.

Concentrations of plasmatic progesterone were determined only in ten agoutis. To avoid

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long periods of capture stress, the animals were distributed into the following groups: 1- Five females were monitored from mating to the 50th day of pregnancy; 2- Another five pregnant animals were monitored from day 50 to 6 hours after delivery.

The blood samples (0.5 mL) were collected two times per week (72 and 96 hours intervals) (Van Aarde & Potgieter, 1986), by saphenous venipuncture, without sedation. A total of 15 samples were taken from each female. A 1 mL syringe with 26 ½-gauge needle (Baas, Potkay, & Bacher, 1976), containing sodium heparin (Liquemine-Roche, Brazil) was used. After collection, the blood was put into a polypropylene tube and immediately centrifuged at 1 000 revolutions per minute for 10 minutes. The plasma was stored at - 20 ºC until assayed.

The progesterone was measured by radioimmunoassay I^{125} in the solid phase (Diagnostic Products Corporation, USA). The assay quality control samples, containing high and low hormone concentrations, were included at the beginning and the end of each assay. Coefficient variations of intra-assay and inter-assay were 11 % and 10 %, respectively. The specificity of the assay for progesterone was 100 % (17-hydroxyprogesterone 0.3 %; 20 α-dihydroprogesterone: 2.0 %), and the sensitivity was 0.02 ng / mL.

After delivery, the onset of ovarian activity in six females was observed daily by colpocytological examination, starting at the seventh day postpartum. Copulation was confirmed by the observation of sperm in the vaginal smear. The postpartum period was monitored four times in five females, and three times in one animal, until copulation. The clinical examination for pregnancy diagnosis (abdominal palpation, auscultation and observation of breast development) was conducted on the 40th day after copulation.

Statistical analyses were performed using SPSS/PC (IBM, USA). Values were expressed as the mean and Standard Deviation (SD). The level of significance throughout the study was 0.05 ($p < 0.05$). Analysis of variance (One-Way ANOVA), in conjunction with Tukey's test, was applied to analyze whether there were significant differences ($p < 0.05$) on hormonal levels during pregnancy; and between the number of suckled offspring and the postpartum estrus period.

RESULTS

The average gestational period observed in this study was 104.04 (SD= 1.31) days, with a range of 101-106 days (Number of cases, N= 26, 78.79 %), and a birth interval of 126.03 days (SD= 18.40) (range of 109 - 184 days).

The plasmatic profile of progesterone during pregnancy showed a progressive increase from the 1st to the 5th week. The higher hormone levels over this period (6.88 ng / mL, SD= 3.01) were detected in the 5th week (29th to the 35th day). This value was similar (One-Way ANOVA, $p > 0.05$) to that observed in the 4th and 6th weeks, but was statistically different (One - Way ANOVA, $p < 0.05$) when compared to the other weeks. After the 6th week there was a progressive decrease in plasmatic progesterone levels. From the 7th week until delivery, there was no statistical difference (One - Way ANOVA, $p > 0.05$) in the levels of this hormone (Fig. 1).

The following gestational problems (N= 7, 21.21 %) were observed: 1- embryonic death after 46 days and 50 days of pregnancy (N= 2), verified by the decrease in the levels of progesterone (Fig. 2); 2- abortions at 73 days and 84 days of gestation (N= 2) (Fig. 2); 3- post-term delivery (106 days, 108 days and 110 days), with consequent dystocia, that result in complicated birthing (N= 3). No perinatal mortalities were observed.

The animals showed a postpartum estrus on average of 12.04 days (SD= 4.29) (7-24 days) (N= 23). It was observed that 80.95 % (N= 19) of copulations during this period were fertile, with the subsequent birth of a young. The gestational period was not confirmed in 9.52 % (N= 2) females showing postpartum estrus, although copulation was observed. No mating was observed in two other animals displaying postpartum estrus. The number

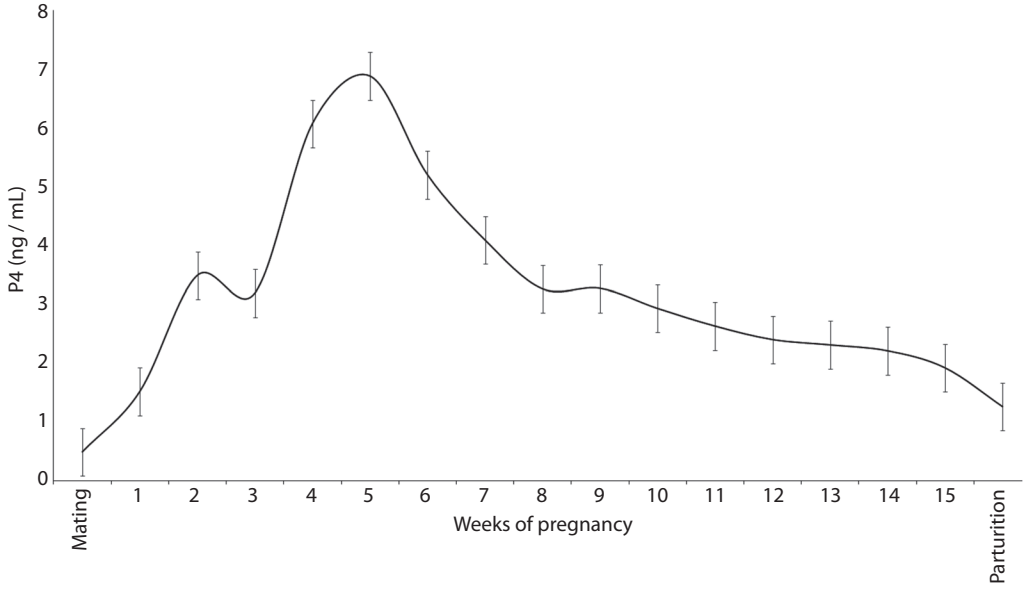

Fig. 1. Mean plasmatic concentrations of progesterone during pregnancy period in agoutis (N = 10).

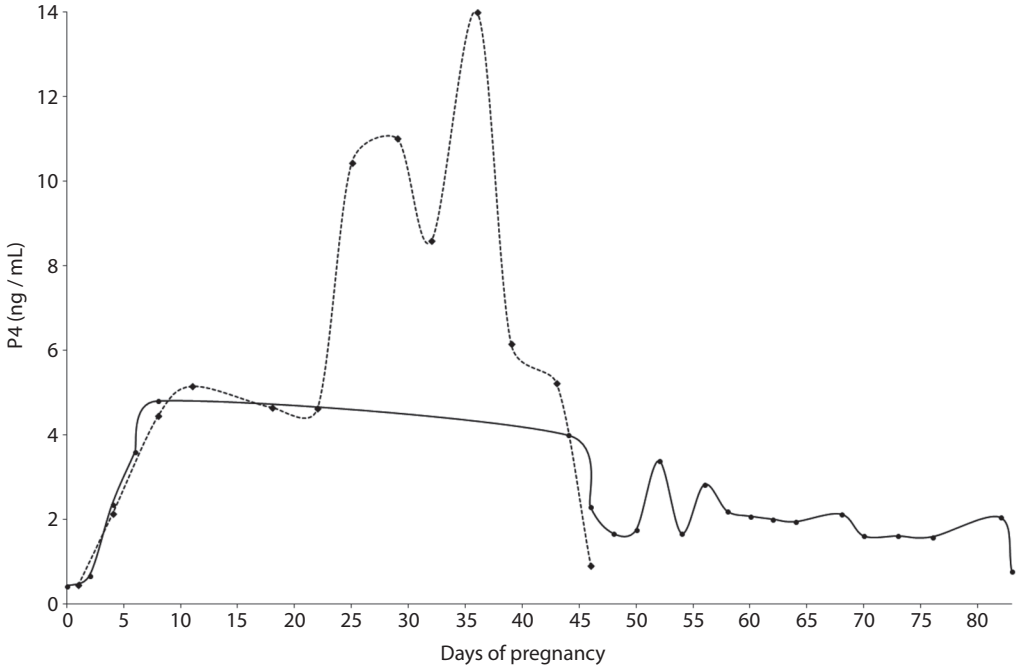

Fig. 2. Decreases in the plasmatic levels of progesterone in agouti, associated to fetal death on the 46th day of pregnancy (dashed line) or to abortion during the 84th day of gestation (solid line).

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of suckled offspring (1-3) had no influence on the postpartum estrus period (One-Way ANOVA, $p > 0.05$).

DISCUSSION

The study of the profile of plasmatic progesterone during pregnancy is an important tool for diagnosis and prognosis of this period, targeting correct reproductive management. Moreover, this knowledge is fundamental and necessary to a better understanding of reproductive physiology of wild animals, which is still very poorly studied.

When compared to other rodent suborders, the hystricomorphs such as the agouti show a long period of gestation (Weir, 1974). This feature could be related to the birth of precocious individuals, adapted to rapid growth and low parental investment. On these aspects, Lange, Abilhôa, Margarido and Monteiro Filho (2003) stated that the offspring of agoutis (*D. azarae*), from larger (lighter) and smaller (heavier) litters, have a similar growth potential, attained at six months of age at approximate final weight. Furthermore, these animals are already able, from the first days of life, to consume solid food, suggesting their autonomy.

The initial stage of pregnancy in agouti is characterized by the progressively increasing progesterone levels until the sixth week. Subsequently, the concentration of this hormone declined until delivery. This secretion pattern is similar to that proposed by Tam (1974) for hystricomorphs.

Despite the similarity in the secretory pattern, the levels of progesterone observed in agouti were lower than those found in *M. pratti* (50 ng / mL) (Rowlands et al., 1970); *C. porcellus* (15 ng / mL in the 15th day of gestation; 329 ng / mL (SD= 14) between the 30th and 45th day; 160 ng / mL (SD= 14), between 51th and 55th days) (Challis et al., 1971); and *G. musteloides* (23 to 60 ng / mL in 15th day of gestation; 513 ng / mL in 26th day; 108 ng / mL a few days before delivery) (Tam, 1973). Moreover, this model of progesterone synthesis was different from that observed in *D. patagonum* (Louis et al., 1986), which showed elevated levels of progesterone after the 4th week (26th day) of gestation, until the time of delivery. In *M. coypus* the highest levels of this hormone was observed between the 14th and 15th week of gestation (12.5 to 20.72 ng / mL) (Jakubička et al., 1989; Barta & Jakubička, 1991); and in *H. africaeaustralis* between the 6th and 12th week (202 ng / mL, SD= 98) of pregnancy (Van Aarde & Potgieter, 1986; Van Wyk et al., 1994). However, during early and mid-pregnancy, the progesterone levels in agouti were similar to those found in *E. dorsatum* (4 ng / mL) (Sweitzer & Holcombre, 1993), and to the maximum values observed after the 60th and 9th days of gestation in *C. paca* (3.03 ng / mL and 3.55 ng / mL) (Ribeiro et al., 2012).

The ovary of pregnant agouti has functional (N= 2) and accessory *corpora lutea*, required for continuous production of hormones (Mayor, Bodmer & López-Bejar, 2011). In general, the synthesis of progesterone in hystricomorphs rodents, during the first stage of pregnancy, occurs in the *corpus luteum*. After this period, in *C. porcellus* and *G. musteloides*, the placenta is responsible for progesterone supplementation (Tam, 1970, 1974). The period between the 4th and 6th week of gestation in agouti, when the progesterone levels are higher, corresponds to the rapid development of the primordial brain vesicle (25th to 30th day of gestation) (Fortes et al., 2013); the first detection of the embryo (18 to 22 days of gestation), fetal heartbeat (25th day of gestation) by ultrasound and the visibility of the placenta (day 21 of gestation) (Sousa et al., 2012). In relation to this endocrine organ, there is a gradual increase up to the 60th day of gestation, after which regression begins (Sousa et al., 2012). This period coincides with the lowest levels of progesterone observed in the present study occurring during the last gestational stage.

The progressive increase in the plasmatic levels of progesterone until the 6th week in agouti shows the great importance of luteal secretion in the maintenance of the endometrium and consequent embryo development during the early pregnancy. This suggests

that from the middle of this period, the placenta might play a more active role in the gestation of this species. This possibility can be verified in the guinea pig (*C. porcellus*), as ovariectomy performed after the middle of this period, does not cause abortion, due to sufficient progesterone produced by the placenta (Hafez, 1980).

Concerning embryonic death and abortion observed in this study, it is possible that endocrine disorders related to maternal age have occurred, as it was the first pregnancy for young females. According to Jainudeen and Hafez (2004), the causes of prenatal mortality may be due to fetal or maternal factors (eg. genetic, endocrine, nutritional, age). Furthermore, in domestic animals, around 25 to 40 % of the embryos are usually lost. It was not possible to clarify the cause of post - term pregnancy observed in this work. However, these problems are generally due to fetopelvic disproportion, abnormal feto positions, absence of uterine contraction, and others. The reproductive failure problems observed in this study were considered as idiopathic causes. Therefore, more studies are necessary for further information.

Agoutis have a postpartum estrus of an average of 12.4 days, which corroborates with Weir (1971). Considering the fertilization rate of 85 % to 100 %, for the species which conceive more than one young (McDonald, 1980), and the observation that 80.95 % of copulations occurred during the postpartum period preceding the normal pregnancies in the agouti, it can be stated that this species is relatively fertile in this period. Similarly, many hystricomorphs exhibit postpartum estrus. However, in *C. porcellus*, *G. musteloides*, *M. coypus* and *Chinchilla laniger*, copulation in postpartum is not always fertile, even though mating of these species has been observed during this period (Weir, 1970, 1973, 1974; Roberts, Maliniak & Deal, 1984; Roberts, Koontz, Phillips & Maliniak, 1987; Iwata, 1989; Jarvis, 1991; Jori, 1998). The presence of postpartum estrus and the subsequent development of another pregnancy in agouti is a positive factor for this species. Because the gestation period is long in the hystricomorphs, the fertile postpartum estrus could ensure a rapid replacement of the population size.

In conclusion, this work contributed to understand the dynamic changes in progesterone levels during the pregnancy in agouti, comparing it to other hystricomorphs and associating it to the main events of embryonic development of this species. This represents a scientific advancement for future implementation of reproductive biotechnologies that aim at conservation of this species. On the other hand, the agouti has a long gestation period, with rapid onset of a fertile postpartum estrus, and produces precocious young, indicating that is a promising species for zootechnical production. However, further investigations are needed to study other endocrine and biological changes in the maternal and feto-placental unit of the agouti, in an attempt to better understand the reproductive failures observed in these animals.

ACKNOWLEDGMENTS

We thank the Federal University of Pará, their technicians for assistance in collecting materials for this research and the International Foundation for Science (Ref. B/2659-1) for supporting this project. The authors thank Martín Alvarez for his help in translation of the abstract into Spanish.

RESUMEN

Perfil de la progesterona plasmática durante durante la preñez y celo posparto del *Dasyprocta prymnolopha* **(Rodentia: Dasyproctidae).** El agouti (*Dasyprocta* sp.) es un roedor histricomorfo encontrado en algunas regiones de América. Es una especie cinegética importante, lo que indica que la caza excesiva es una amenaza para su conservación. Muy poco es lo que se conoce acerca de la biología de los animales silvestres, en relación al conocimiento acumulado sobre los domésticos. Por lo tanto, el estudio sobre la reproducción de los animales silvestres se hace necesario e imprescindible para el manejo y conservación de la especie como recurso natural. En concreto, los estudios relativos a la monitorización hormonal son una herramienta básica para la investigación en biotecnología reproductiva moderna. No hay información sobre los cambios de progesterona durante la preñez de *Dasyprocta* sp.

en comparación con otros histricomorfos. El objetivo de este estudio fue describir el perfil de la progesterona plasmática durante la preñez, y reportar el reinicio del ciclo ovárico después del parto. Para ello, se utilizaron 18 agoutis de rabo negro (*Dasyprocta prymnolopha*) nacidos en cautiverio. Fueron estudiadas una o más preñeces consecutivas. La copulación fue confirmada por la presencia de espermatozoides observados en examen colpocitológico. Las muestras de sangre se recogieron dos veces por semana, y las concentraciones de progesterona se determinaron en 10 individuos mediante radioinmunoensayo. Se observó el inicio de la actividad ovárica diaria por examen colpocitológico, comenzando en el día séptimo posparto. El período gestacional observado en este estudio fue de 104.04 días (SD= 1.31, rango entre 101-106 días, N= 26), y el intervalo entre los nacimientos fue 126.03 días (SD= 18.40, rango entre 109-184 días). El perfil plasmático de la progesterona durante el embarazo mostró un aumento progresivo de la 1ª a la 5ª semana. Los mayores niveles de progesterona durante este período (6.88 ng / mL, SD= 3.01) se detectaron en la quinta semana. Este valor fue similar (ANOVA de um factor, $p > 0.05$) al observado en las semanas cuatro y seis, pero fue estadísticamente diferente (ANOVA de um factor, $p<0.05$) en comparación con las otras semanas. Después de la sexta semana hubo una disminución progresiva en los niveles plasmáticos de progesterona. Los animales mostraron un estro posparto de 12.04 días (SD= 4.29, rango entre 7-24 días, N= 23). Se observó que el 80.95 % (N= 19) de cópulas durante este período fueron fértiles. Este trabajo contribuyó a la comprensión de los cambios dinámicos en los niveles de progesterona durante la preñez del agouti. Sin embargo, se necesitan más estudios para una mejor comprensión de otros cambios biológicos y endocrinos, y en la unidad materna y feto-placentaria del agouti.

Palabras clave: histricomorfos, agouti, gestación, posparto, progesterona.

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