Synchronization of estrus in paca (Cuniculus paca L.): possible impacts on reproductive and productive parameters

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Abstract
Estrus synchronization is a reproductive biotechnology used to improve artificial insemination or pairing through the manipulation of the estrous cycle at a desirable time. Employing this technique in captive pacas (Cuniculus paca L.) is important because it creates expectation of meeting the demand for paca meat and, consequently, reduces poaching. Thus, this research aims to verify the effect of a progestogen implant associated with two doses of eCG on the synchronization and induction of fertile estrus. Twenty-seven adult pacas were used, 18 non-pregnant females and nine males, divided into three groups. G1 and G2 females groups (treatments) received 1.5 mg Norgestomet and were injected intramuscularly, seven days later, with 0.13 mg of prostaglandin. After 24 hours the implants were removed and the animals immediately received 25 IU and 50 IU of ECG intramuscularly, respectively. The mating of the three groups took place on the same days. G3 females’ group (control) showed estrus on different days after D0. Females under treatment displayed estrus only after removing the implant (D8). G1, G2, and G3 pregnancy rates were 100%, 66%, and 50%, respectively. Regarding births per parturition, 100% of G1 and G3 produced one offspring, while 50% of G2 produced two. Progestogen in the form of subcutaneous implants was effective in mimicking the luteal phase of the estrous cycle. After removal, implants favored the occurrence of a fertile estrus. As a conclusion, further studies must be conducted in order to establish in-depth possible association between 50 IU of eCG, and the occurrence of twin pregnancies.

Keywords: Heat. Synchronization. Progestin. Rodent.

Resumo
A sincronização é uma biotécnica reprodutiva que melhora a porcentagem de cobertura por meio da manipulação do ciclo estral. Empregando esta biotécnica em pacas de cativeiro (Cuniculus paca L.) é importante, pois cria-se a expectativa de que a demanda pela carne seja atendida e a caça ilegal diminua. O objetivo da pesquisa foi verificar o efeito de implantes de progestágenos associados a duas doses de gonadotrofina coriônica equina (eCG) na sincronização e indução de cios férteis de pacas. Foram utilizadas 18 fêmeas não prenhas e nove machos, divididos em três grupos. Fêmeas do G1 e G2 foram submetidas a implantes com 1,5mg de Norgestomet e, sete dias após, 0,13mg de prostaglandina via intramuscular (IM). No dia 8 (D8), foram retirados os implantes e G1 e G2 receberam 25 UI e 50 UI de eCG, IM, respectivamente; G3 foi o controle. O pareamento nos três grupos aconteceu nos mesmos dias. As fêmeas do G3 apresentaram cio alguns dias após o dia zero (D0). Fêmeas que receberam tratamento apresentaram cio só após a retirada do implante no dia 8 (D8). As taxas de prenhez de G1 e G3 atingiram 100%, 66% e 50%, respectivamente. Em relação a filhotes por parto, 100% do G1 e G3 produziram uma cria, enquanto 50% do G2 produziram duas crias. O progestágeno do implante foi eficaz em mimetizar a fase lútea do ciclo estral. Após a remoção, o tratamento hormonal favoreceu a ocorrência de cio fértil. Outros estudos devem ser realizados a fim de estabelecer uma possível associação entre 50 UI de eCG e a ocorrência de gestações gemelares.

Introduction

The paca (Cuniculus paca L.), in Latin America, is among the most intensively hunted species due to its culinary lusciousness (MAYOR et al., 2013). In addition, being a small animal easily adapted to captivity and interacting spontaneously with humans, it is often captured to become a pet (MONTES, 2005). These aspects, associated with inappropriate land use and forest mismanagement, have almost caused extermination by a drastic animal reduction in some regions.

Paca meat production, already held in some farms and facilities, has created the perspective of a met demand, which eventually decreased poaching (SMYTHE; GUANTI, 1995; NOGUEIRA FILHO; NOGUEIRA, 1999). However, low reproductive rates characterized by females, which rarely give two offspring per birth, almost twice a year, may be discouraging factors for potential breeders.

On the other hand, the gestation period has commonly been the most controversial parameter between authors, reaching a difference between reported periods of up to 76 days. Bonilla-Morales et al. (2013), for example, cites a range of 76-101 days, while Scherf (1997) and Mayor et al. (2013) reported variations of 138-173 days and 135-155 days, respectively.

According to Lameira (2002), biotechnology and the implementation of appropriate management techniques allow genetic selection, which will result in increased reproductive and productive efficiency of this animal.

Estrus synchronization is a reproductive biotechnology used to improve artificial insemination or pairing, as well as the birthing process, through the manipulation of the estrous cycle at a desirable time (HAFEZ, 2004). Through this technique, the breeder can focus on a timely labor. For instance, during low rainfall periods, pneumonia incidence and endoparasite infestations of wild animal in captivity diminishes while herds remain more homogeneous, which facilitates its management and sale.

The pharmacological regulation of the estrous cycle in domestic animals implies lifespan control of the corpus luteum. Therefore, progesterone (P4) or other progestogens are used to control ovulation time in goats, cows, sheep, and mares (HAFEZ, 2004). Progesterone, natural or synthetic, with or without pituitary gonadotropins, and extra-pituitary prostaglandin (PGF2a) have been successfully used in the synchronization and induction of fertile estrus in different species (DIAS et al., 2001; ROCHA et al., 2007; NOGUEIRA et al., 2008; MAIA JÚNIOR, 2009; MELLO; PINTO-NETO, 2009; MONREAL et al., 2009).

Currently, estrus and ovulation can already be synchronized in cyclic animals by combining progestin and luteolytic agents. Estrogen and PGF2a, or its analogue cloprostenol, are the most used (JAINUDEEN et al., 2004).

Associated to progestin and PGF2a, gonadotropins have further improved results in terms of pregnancy, since the equine chorionic gonadotropin (e.g.) stimulates the development of ovarian follicles (MONREAL et al., 2002; HAFEZ, 2004). An advantage of ECG linked to synchronization protocols is related to the hormone opportune administration during progestogen implant removal, thus saving labor for animal handling as compared to other protocols (MOREIRA et al., 2007).

In Cuniculus paca, no reports about animal response to hormone therapies used in ovulation induction protocols and estrus synchronization exist, nor about dosages needed to create an effective fertile estrus. Likewise, it is undefined whether this practice has influence on other reproductive or gestational parameters.

Therefore, this study aims at testing hormone protocols to induce and synchronize estrus in fertile pacas.

Materials and Methods

Study area

This study was approved by the Brazilian Ministry of Environment-MMA, Chico Mendes Institute for Biodiversity Conservation, under number 24669-1 (authorization for activities with scientific purpose), and developed in the wild animal breeding of Experimental Farm Catuaba, University Federal do Acre, municipality of Senador Guiomar, 30 km from BR - 364 highway (10°04’S and 67°37’W). The Climate in the region is typical of equatorial Amazon with temperatures ranging from 24.5°C to 32°C and relative humidity of 80% to 100 % (ACRE, 2006).
Animals and treatment

Twenty-seven adult pacas (18 non-pregnant multiparous females and 9 males) were used. Animals were placed in nine bays, with two females and one male each, later divided into three experimental groups: G1 and G2 (treatments), and G3 (control). Each group consisted of three animals per bay.

The diet of each animal contained fruits (386 g), grains (119 g), tubers (112 g), and vegetables (109 g), and was complemented with mineral supplement (mineral salt, base 40P, Real H brand) and water ad libitum.

G1 and G2 females received implants with 1.5 mg of Norgestomet (Crestar, Intervet) in random days of the estrous cycle – half of the commercial presentation according to what has been described by Aramburo et al. (2006), which were inserted subcutaneously and dorsally 4 cm from the paraspinal region. In all groups, the time of progestagen implantation was considered day zero (D0). Seven days later, 0.13 mg of prostaglandin (Ciosin, Coopers) was administered intramuscularly (i.m.). Finally, implants were removed 24 hours after the last treatment (D8). Immediately after removing the implants, females of G1 and G2 received 25 IU and 50 IU ECG (5000 Novormon), i.m., respectively. Males were introduced in the three groups on the day of progestin implant (D0).

When placing implants, each female was sedated with 1 ml of 1% acepromazine (Acepran, Univet), i.m. After cutting the animal’s hair, implant area was disinfected with povidone iodine and a subcutaneous anesthetic block was conducted with lidocaine hydrochloride (2% lidocaine, Bravet), followed by 3 cm skin incision for implantation. Cotton thread 2-0 (Ethicon) was utilized for suturing subcutaneous tissue where implant remained, exposing it 3 cm. After healing, such excedent allowed implant to remain in control during the experiment (Figure 1). Finally, a herbal healing repellent product was applied on surgical wound area (copaiba oil). The process was conducted in 5 minutes.

In order to determine estrus cycle stages and mating occurrence in female paca, after vulvar hygiene, a vaginal sample collection was performed with swabs to analyze cellular components and/or detect sperm presence in smears (Figure 2). This procedure was initiated in G3 on D0, and then every 48 hours until pairing confirmation. For G1 and G2, observations were made every 48 hours until D8, and then every 24 hours until coupling confirmation.

The material obtained from the swabs was analyzed regarding color and texture. Afterwards, the smear was duly carried out by means of identified histological slides, fixed in 95% alcohol, stained according to the Giemsa technique and examined under a light microscope (Olympus CH30), with 10x and 40x objectives. Convex ultrasound transducer of 7.5 MHz (Aloka, model SSD-500v – Aloka Co. Tokyo, Japan) was performed sixty and ninety days after pairing to confirm gestation.

For obtaining the progesterone levels in the plasma, before and after the implant, blood samples from females were collected (3 mL) by venipuncture of the cephalic vein, scalps with disposable 21 G and 5 mL syringes containing EDTA were used.

Properly identified samples were kept refrigerated in ice coolers (maximum 3 hours) until being subjected to centrifugation at 900 g for 20 minutes. Plasma samples were placed in polypropylene vials and stored at -20°C until measurement. Progesterone concentrations were determined according to the radioimmunoassay described...
Results and Discussion

In this research, the progestin implant (1.5 mg Norgestomet) in females of the G1 and G2 groups was effective in the suppression of estrus manifestations and possible ovulation until withdrawal (D8); in G3 females undergoing no treatment, estrus appeared on different days after D0. Comparing estrus appearance in all groups after D0, the statistical difference between treated and untreated females was determined (p < 0.05). However, such difference was not observed in treated groups (p > 0.05). These data are compliant with Hafez (2004), who stated that among other actions on reproductive physiology progesterone plays a key role in the hormonal regulation of estrous cycle, causing both heat inhibition and pre-ovulatory LH peak, beyond synthetic progestin blockage of LH secretion from pituitaries. Africander et al. (2014) and Mendonça et al. (2007) reported a connection of synthetic progestin to receiver similar to the natural molecule, thereby promoting competition in the binding target. This fact would cause the disruption of the estrous cycle.

Aramburo et al. (2006) managed to induce the estrus cycle in six mountain pacas (Agouti taczanowiskii), a similar species, using 1/4 Norgestomet. The effectiveness of the treatment in the synchronization of three females was noted, considering cycle onset the day of vaginal canal opening. In 12 female animals receiving hormonal treatments, results were adequate because all of them entered estrus. Moreover, 11 (91.66%) of them were copulated by males, with estrus in G1 in 100% of the cases (6/6), and in G2 in 83% of the cases (5/6). Due to the lack of similar studies in Cuniculus paca for comparative purposes, outcomes were checked by the physiology of other species. Some studies with sheep employing similar induction/synchronization protocols displayed percentages of estrus occurrence ranging from 100% to 88.5% (BICUDO; SOUSA, 2003). Their use may also imply such benefits on paca husbandry.

The first female animals manifesting estrus were those receiving 50 IU eCG. For pacas, literature on this topic is nonexistent. In other species, Evans and Robinson (1980) found a negative correlation between the used dosage of eCG and the time of estrus presence after treatment, with a decreasing of 14 hours at higher dosages. Therefore, such differences found in other investigations suggest the need for a broad approach to the determination of whether a contrary and positive effect happens in pacas by increasing eCG dosage.

All G3 females came into estrus, but only in three an effective intercourse was possible. In this case, the pacas may have presented silent heat, that is, hormonal events characterized estrus manifestation, but external signs are not strong enough to be noticed by males. Given these findings, we emphasized that hormonal treatment in G2 and G3 helped heat demonstration and observation by males, which led to an increase of mated and pregnant females in such groups.

The G3 untreated females also developed a shortening of estrous cycle stages, as well as a high estrus manifestation between the first and the thirteenth day of pairing. This behavior seems to indicate a species susceptibility to male effect.

In other species, male presence in a female group can anticipate the start of breeding season (WEIR, 1970) and motivate estrus synchronization through a quick stimulus for GnRH-LH release (MORAES et al., 2008), which can result in various pregnant females at the same time (NOGUEIRA FILHO; NOGUEIRA, 1999; BRIEVA, 2001). However, this effect demands a previous period of sexual isolation (MORAES et al., 2008).

Such conditions have not been applied in this experiment due to its initial design. Nonetheless, we need to identify whether there really is a modulating male effect over heat presentation in this species. Moreover, in the case of such condition presenting itself, a comparison between estrus modulation generated by hormonal treatment and the male effect must to be performed.

The average progesterone concentrations observed in various cycle phases of the paca before hormone implantation were 1.69 ± 1.26 ng/mL in proestrus (n = 4); 0.31 ng/ml in estrus (n = 1), 1.62 ± 1.39 ng/ml in metestrus (n = 4), and 0.88 ± 0.70 ng/ml in diestrus (n = 3). These findings, including the large standard deviations, are similar to those described by Guimarães et al. (1999), Pérez (2001) and Pérez and Baz (2006).

Moreover, Reis (2013) measured metabolites in paca feces (Oryctolagus PACA) and noted values ranging...
from 0.37 to 7.9 ng/g throughout the cycle progestogen. Nevertheless, the progestin fluctuations characteristic of this phase were not present. Ribeiro et al. (2012) compared progesterone levels in chinchillas (Chinchilla laniger Gray, 1831) and found significant changes in hormone concentrations, especially during pregnancy. Likewise, these authors described an increased progesterone concentration during early pregnancy in green acouchi (Myoprocta pratti, Pocock, 1913), which remained beyond the half and declined at the end.

Finally, the average value for progesterone at the implant withdrawal was 1.35 ± 0.78 ng/ml, with a maximum of 2.87 ng/ml, and a minimum of 0.31 ng min/ml. Mauer et al. (1975) reported that progesterone-impregnated intravaginal devices increase P4 blood levels in < 1ng/ml for 6 ng/ml, within 90 minutes after its insertion, declining from 1 to 3 ng/ml after 14 days.

Due to the lack of similar reports, the above findings have been comparatively described in other species. Custer et al. (1994), using progesterone-releasing intravaginal device (PRID) in cows, observed an increasing P4 serum concentration of about 25% twenty-four hours after application, and a progressively declining subluteal phase concentration (< 2 ng/ml) until removal. Siros and Fortune (1990) obtained P4 from 1 to 2 ng/ml P4 after placing, for about nine days, a vaginal insertion device containing progesterone (CIDR-Controlled Internal Drug Release Devices) in heifers.

Cytological analyses showed intermediate cells with nucleoprotein pigments next to estrus expression, as well as intermediate cell pre-ovulatory type (Figures 3 and 4). The influence of progesterone and estrogen on the dynamics of exfoliated vaginal cells (EVC) has already been studied in other species (PÉREZ-MARTÍNEZ et al., 1999; RIBEIRO et al., 2007).

Pérez-Martínez et al. (1999) concluded that EVC are highly indicative of progesterone effects, which can be a useful tool to detect hormonal treatment response. These results are consistent with those found in the present research.

Ultrasound and cervical cytology, performed sixty and ninety days after pairing, allowed the identification of six (100%), four (66%), and three (50%) pregnant females in G1, G2, and G3, respectively. When considering all animal groups, receiving or not treatment, pregnancy rates were 83.3% and 50%, respectively. Concerning G2, despite the fact that all females displayed estrus, one female was not completely copulated by presenting a low quantity of sperm in the smear test. In the absence of standard references for pacas, results were compared to protocols applied in other animals, which proved being superior to those obtained by Dias et al. (2001), Carrijo Junior and Langer (2006), and Fonseca et al. (2008).

Regarding the female ability to achieve term pregnancy, out of six females from G1, five gave birth and one aborted after completing 90 days of pregnancy. This miscarriage was probably due to its aggressive and irritable behavior not only during parturition, but also to bay peers and handlers.

In G2, the four mated females delivered, but in two of them twin births occurred (Figure 5), giving six babies in total. It means an increase of 50% on expected births.

Figure 3 – Intermediate cells with nucleoprotein pigment, Cuniculus paca. Col. Harris-Shorr technical. 400x. Experimental Farm Catuaba, University Federal do Acre

Figure 4 – Pre-ovulatory cells of Cuniculus paca. Col. Harris-Shorr. 400x. Experimental Farm Catuaba, University Federal do Acre
These results differ from those reported by Nogueira et al. (2006), Oliveira et al. (2007), and Bonilla-Morales et al. (2013), who underscore uniparous reproductive trend of giving one offspring per birth. Therefore, investigating whether there is an association between the applied treatment and multiple births in paca remains as an impending need. Finally, the three paired females from control group (G3) completed the pregnancy term.

The mean and standard deviation for the female gestation period in G1, G2, and G3 were 154 (± 3.4), 156.75 (± 2.0), and 152.33 (± 0.47) days, respectively. These results are higher than those presented by Bonilla-Morales et al. (2013) – 97 to 101 days; Hosken (1999) – 116-135 days; Colombia (1998) – 120 days; Oliveira et al. (2003) – 135 to 139 days; and similar to Smythe and Guanti (1995) – 157 days; Rengifo et al. (1996) – 145 to 155 days; Lameira (2002) – 142-154 days and Guimarães et al. (2008) – 148.6 ± 4.8.

Regarding offspring weight at delivery time, outcomes were 860 (± 134), 716.67 (± 55), and 883.3 (± 23.5) g for G1, G2, and G3, respectively. No significant difference (p > 0.05) for birth weight after hormone treatment (25 and 50 IU eCG) was found. However, a significant difference appeared with respect to untreated groups (G3 and G1, G2 and G3) (p < 0.05). This fact can be explained by the occurrence of twin births in treated females, giving birth to paca babies with lower weights. Nonetheless, such results were similar to those observed by Guimarães et al. (2008) regarding the average birth weight for females and males: 605.9 ± 736.7 ± 108.4 and 87.5 g. Ribeiro and Zamora (2008), and Oliveira et al. (2007) at 787 ± 51.23 g and 741.14 grams, respectively; and superior to Hosken (1999), 500 to 650 g, and Santos et al. (2005), 615 and 617 g for males and females, respectively.

**Conclusion**

The progestin implant in females of G1 and G2 was effective to suppress estrus manifestations and possible ovulation and favored the occurrence of fertile estrus accompanied by physical and behavioral manifestations. Likewise, females treated had higher pregnancy rates and increase of 50% on expected births. Before such relevant findings as twin birth occurrence, the authors recommend the development of more studies to inquire a possible association between 50 IU eCG dose and this outcome.

**Conflict of Interest**

None declared.

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