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Single injection of eCG/hCG leads to successful estrous synchronization in the collared peccary (*Pecari tajacu* Linnaeus, 1758)

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A R T I C L E I N F O

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A B S T R A C T

The establishment of protocols for the control of the ovarian function of collared peccaries is recommended for the development of assisted reproductive techniques. The goals were to (1) compare a gonadotropin combination with prostaglandin analogue to synchronize timing of onset of estrus among animals, and (2) elucidate the effects of the most desirable protocol for performing an artificial insemination study and macroscopic evaluation of the ovaries. Three of five females treated with a double administration of 120 μg prostaglandin (cloprostenol) at a 9-day interval expressed symptoms of estrus 9 days after the second injection. One female presented estrus after 6 days, whereas other did not respond to the treatment. All females (5/5) treated with a single dose containing 400 IU eCG and 200 IU hCG manifested estrus 6 days after the hormone injection. In a second experiment, ten females that were estrous synchronized using eCG/hCG, were artificially inseminated with fresh semen and monitored for pregnancy every 30 days. Although there was no detection of fetuses by ultrasonic examination, seven females (7/10) had greater than basal progesterone values for 60 days after the treatments were imposed. Ovaries from two females treated with eCG/hCG were collected 6 days post-injection. There was confirmation of an ovarian stimulation as a result of the presence of 88 and 25 antral follicles, as well as three and eight hemorrhagic structures in ovaries of each female, respectively. It, therefore, is proposed that eCG/hCG can be used as an effective treatment for estrous synchronization in collared peccaries.

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1. Introduction

The collared peccary (*Pecari tajacu*) is a suiforme that inhabits the Americas, with a population globally classified as being at least concern (Gongora et al., 2011). Excessive poaching associated with deforestation and expansion of large-scale ranching and agriculture, however, are affecting the survival of this species. As a result, peccaries are now extinct in North Argentina (Gongora et al., 2011) and populations are decreasing in the Atlantic Forest (Desbiez et al., 2012). The species contributes to the spread of vegetation and seed dispersion through the consumption of several species of plants (Pérez-Irineo and Santos-Moreno, 2016), and is also prey for large carnivores (Scognamillo et al., 2003). In addition, peccaries are a source of income and animal protein for subsistence in many human communities in the Amazon and other places in Latin America (García-Marmolejo et al., 2015; Ripple et al., 2016). To contribute to population management and conservation, assisted reproductive techniques (ART) are being developed for this species (Souza et al., 2016; Lima et al., 2018).

The development of artificial insemination (AI) allows for the exchange of genetic material as semen can be collected from different regions, especially because the freezing-thawing techniques for collared peccary semen have been established (Castelo et al., 2010; Souza et al., 2016). To facilitate the development of AI programs, it is recommended to control ovarian function with the administration of exogenous hormones. The estrous cycle in collared peccaries lasts 21.0 ± 5.7 days, ranging from 15 to 27 days (Maia et al., 2014a). During natural estrus, actual time of ovulation is difficult to determine, therefore, there is a lack of synchronization in time of breeding of females within a herd (Mayor et al., 2007a; Maia et al., 2014ab). To allow for AI at a synchronized time among females, development of treatments for the control of both luteal and follicular functions that can lead to the control and synchronization of the time of ovulation without the requirement for estrous detection are needed (De Rensis and Kirkwood, 2016). Maia et al. (2014b) previously tested a protocol for estrous synchronization of the collared peccary based on a prostaglandin analog, cloprostenol, which provided an effective (80%), but delayed response (9.5 ± 0.5 days post-administration) as compared with is desired. In the domestic pig, the most closely related animal model, the administration of equine (eCG) and human chorionic gonadotrophins (hCG) as a single does induces rapid estrous induction (generally after 5 days on average) and can be used to synchronize time of ovulation among females (Stefan et al., 2015).

The efficiency of use of estrous synchronization protocols can be evaluated through techniques usually adopted for estrous monitoring through hormone assays, vaginal cytology, and ultrasonography (Sontakke, 2018). in vivo assays following AI are important for assessing the success of an estrous synchronization program (Knox et al., 2000). The goals of the present study were to (1) study the efficiency of different treatments for estrous synchronization in collared peccaries, and (2) further elucidate the effects of the most effective protocol through an AI study and macroscopic evaluation of ovaries.

2. Material and methods

The ethics committee of the UFERSA, Mossoró, Brazil, approved experimental protocols and animal care practices for this study (Process n° 23091.000250/2011-34). Procedures were also authorized by the Chico Mendes Institute for Biodiversity (SISBIO Nº 37329). The study was conducted at the Center of Multiplication of Wild Animals (CEMAS – UFERSA), located in the north-east of Brazil (Mossoró, RN, Brazil; 5°10′S, 37°10′W), registered as a scientific breeding center at the Brazilian Institute of Environment and Renewal Resources (IBAMA Register Nº 1478912).

2.1. Animals

Ten apparently healthy, mature, and non-pregnant female collared peccaries aged 2.5 ± 0.5 years (ranging from 2 to 3 years), weighing 20.6 ± 1.1 kg (ranging from 19 to 22 kg), and known to have had at least one pregnancy before the start of the experiments were used. In addition, ten mature males, with a mean age of 1.9 ± 0.3 years months (ranging from 1,5 to 2,5 years) and weighing 20.1 ± 0.5 kg (ranging from 19 to 21 kg), were used for estrous detection or as semen donors.

Animals were maintained in natural lightning conditions (~12 h) and divided into groups of five animals housed in paddocks (20 × 3 m) with a 3 × 3 m covered area. Females were fed an isocaloric (3 300 kcal/kg) and isoproteic (14% protein) diet consisting of corn (79.8%), soy bean meal (15.4%), wheat bran (1.45%), calcium (2.6%), and a vitamin (0.2%) and mineral premix (0.05%), supplemented with tropical fruits, such as melon. Water was provided ad libitum.

2.2. Anesthesia

For all procedures involving animal handling, animals were previously fasted for 12 h. Subsequently, animals were physically restrained using a hand net and anesthetized with intravenous injection of propofol (Propovan®, Cristalia, Fortaleza, Brazil) given as a single bolus (5 mg/kg) (Souza et al., 2009). Physiological metrics were monitored during all procedures, including body temperature, pulse, and respiratory frequency.

2.3. Monitoring reproductive status

For the identification of stage females estrous cycle at the beginning of the experiment, there was quantification of 17β-estradiol and progesterone concentrations in blood 3 days before and on the day which the experiment was initiated. From the day of drug administration (Day 0) to day 21, females were monitored every 3 days throughout the study for 17β-estradiol blood concentrations, vulval physical characteristics, vaginal cytology, and ultrasonography.

The stage the estrous cycle for females was identified based on the concentrations of blood progesterone (ng/mL) and 17β-estradiol profile (pg/mL) previously established for collared peccaries (Mayor et al., 2007b). Blood samples (3.0 mL) were collected from the cephalic venipuncture into silicon tubes and centrifuged at 200 *g* for 15 min for 2 h. The serum was subsequently separated and stored at −18 °C. The 17β-estradiol assays were conducted using a commercial kit (Max-Planck-Ring D-21 65 205, Human GmbH, Wiesbaden, Germany) for enzyme-linked immune sorbent assay (ELISA), as previously described for this species (Mayor et al., 2007b).

External symptoms of estrus were monitored by assessing vulvar appearance as reported by Mayor et al. (2007b). This evaluation included the observation and subsequent description of the labia and commissures of the vulva, and mucus in the vestibulum vaginae. Observation of reddish and tumescent vulval appearance and fluid vaginal mucus was recorded. The external vaginal opening was also assessed and classified as being open or closed. The vaginal opening was described as open if the cotton-tipped swab used for obtaining vaginal smears could be easily introduced into the vagina.

For vaginal cytology, a cotton-tipped swab was introduced into the caudal vagina, and the cells were transferred to a glass slide and stained with a Diff-Quick stain (Instant-Proov®, Newprov, Pinais, PR, Brazil). Cells (n = 200) were counted using light microscopy (x400), and results were recorded as the proportions of parabasal, intermediate, and superficial cells counted. The appearance and the relative proportions of cell types were used to determine the stage of the sexual cycle in a similar way to the method described by Mayor et al. (2007b).

Ovaries were examined by transabdominal ultrasonography using a 6–8 MHz micro convex array transducer and a B-mode, real-time, portable equipment (Aquila Vet; Pie Medical, Nutricell, Campinas, Brazil). After anesthesia, animals were positioned in lateral recumbence, and acoustic gel was applied in the area to be examined. Ovaries were evaluated for echoic appearance and texture. Identification (presence or absence) and measurements of the ovarian structures, follicles, and corpus luteum were recorded. Ovarian diameters were measured using electronic cursors integrated into the ultrasonic equipment.

2.4. Experiment 1 – Estrous synchronization

Females were divided in two groups of five individuals. The first group received two intramuscular injections of 120 μg cloprostenol (Ciosin®, Schering-Plough-Coopers, Brazil) administered at 9-day intervals, as previously proposed for this species (Maia et al., 2014b). The second group received an intramuscular single injection of a gonadotropin combination containing 400 IU eCG and 200 IU hCG (PG600®, Intervet, São Paulo, Brazil), as previously used in the domestic pig (Stefan et al., 2015).

Immediately after hormone administration, a stud male was introduced into each female group to monitor their sexual behavior, as describe by Silva et al. (2016). One male and five females were kept together for 21 days. Females were subsequently maintained as isolated individuals for 5 months to determine the occurrence of pregnancy and subsequent parturition.

2.5. Experiment 2 – Artificial insemination study

The ten females subjected to the administration of the gonadotropin combination (400 IU eCG and 200 IU hCG – PG600®, Intervet, São Paulo, Brazil) were assessed to evaluate the effectiveness of the synchronization protocol used. On the day of hormone administration (Day 0), the females were monitored every 3 days as previously described; however, 17β-estradiol and progesterone profiles were determined using an amplified chemiluminescence technique (Immunodiagnostic VITROS® ECiQ; Johnson & Johnson do Brasil LTDA, São Paulo, Brazil), as described by Bartoskova et al. (2014).

Females were subjected to two consecutive AIs, using fresh ejaculates, 6 and 8 days after gonadotropin administrations. On the day of insemination, semen was obtained by electro-ejaculation and immediately evaluated for volume, concentration, sperm motility, vigor (0–5), morphology, viability, and osmotic response. Ten mature males were used as semen donors (one ejaculate per female and per insemination). Values for semen variables (Table 1) were in the normal range for the species (Peixoto et al., 2012). For each insemination, the contents of a complete ejaculate of a single male was inserted using a syringe.

On the day of AI, females were anesthetized, positioned in dorsal recumbence, and the vulvar region was cleaned. Blood was collected to quantify the 17β-estradiol and progesterone serum concentrations. For the AI procedure, a pipette, conventionally used for AI in cattle, was carefully inserted through the vagina until resistance was felt, indicating that the end of the pipette being inserted was positioned at the caudal area of the cervix. The syringe containing semen was subsequently coupled to the outside edge of the

Table 1
Semen quality from collared peccaries (n = 10 males) collected by electro-ejaculation and used for intravaginal artificial insemination (AI). Values are expressed as mean ± SEM.

	First AI	Second AI
Volume (mL)	2.2 ± 0.9	5.9 ± 3.5
Vigor (0 – 5)	5 ± 0	4.8 ± 0.1
Motility (%)	87.1 ± 3.6	85.7 ± 6.7
Concentration x 10^6 (sperm/mL)	467.1 ± 191.7	328.6 ± 169.2
Sperm viability (%)	95.6 ± 5.5	75.5 ± 3.5
Osmotic response (%)	101.9 ± 3.1	81.4 ± 2.3
Normal spermatozoa (%)	89 ± 3.0	83.2 ± 14.8

pipette and the semen was deposited in the cranial vagina or close to the caudal lumen of the cervix. Subsequently, the female hind limbs were maintained in a raised position for 10 min, to reduce semen reflux. The occurrence of bloody discharge during insemination was recorded.

During 60 days after AI, females were monitored daily for sexual behavior relative to estrous manifestation (Silva et al., 2016), and at 30 and 60 days after AI, a uterine ultrasonography was performed. The uterus was evaluated for the size, echogenicity, echotexture, and presence of luminal contents (Maia et al., 2014b). On these occasions, there was also blood collection for 17β-estradiol and progesterone quantification using chemiluminescence as previously reported (Bartoskova et al., 2014).

2.6. Experiment 3 – Macroscopic examination of ovaries

There was evaluation of the effect of gonadotropins on the ovaries of two mature females that previously received a single administration of 400 IU eCG and 200 IU hCG (PG600®, Intervet, São Paulo, Brazil). Six days later, females expressed symptoms of estrus in the form of an open, swollen, and hyperemic vulva, and vaginal mucus, and were then euthanatized and ovarian structures were examined. Ovarian follicles were evaluated for diameter and were classified as small (< 0.3 cm), medium (0.3 – 0.5 cm), or large follicles (> 0.5 cm) as described by Abeydeera (2002). The presence of hemorrhagic bodies and corpora lutea were also recorded.

2.7. Statistical analysis

Data were expressed as mean and standard error (SEM) and analyzed using StatView 5.0 software (SAS Institute Inc., Cary, USA). Values for 17β-estradiol concentrations in blood, proportion of superficial cells on vaginal cytology, diameter of follicles, or number of follicles per ovary were compared using the Fisher's protected least significance difference (PLSD) test ($P < 0.05$). The efficiency of the protocols for estrous synchronization was compared using Fisher's exact Test ($P < 0.05$).

3. Results

3.1. Experiment 1 – Estrous synchronization

All experimental females ($n = 10$) were in the luteal phase on the first treatment day (Day 0). A large individual variation related to the 17β-estradiol profile was observed among females from both treatment groups (Fig. 1A,C); however, there were no significant differences between treatments related to estrous occurrence, 17β-estradiol peak values, proportion of cells observed using vaginal cytology, number of follicles per ovary, or dimensions of follicles (Table 2). Furthermore, the efficiency of the treatments (80% for

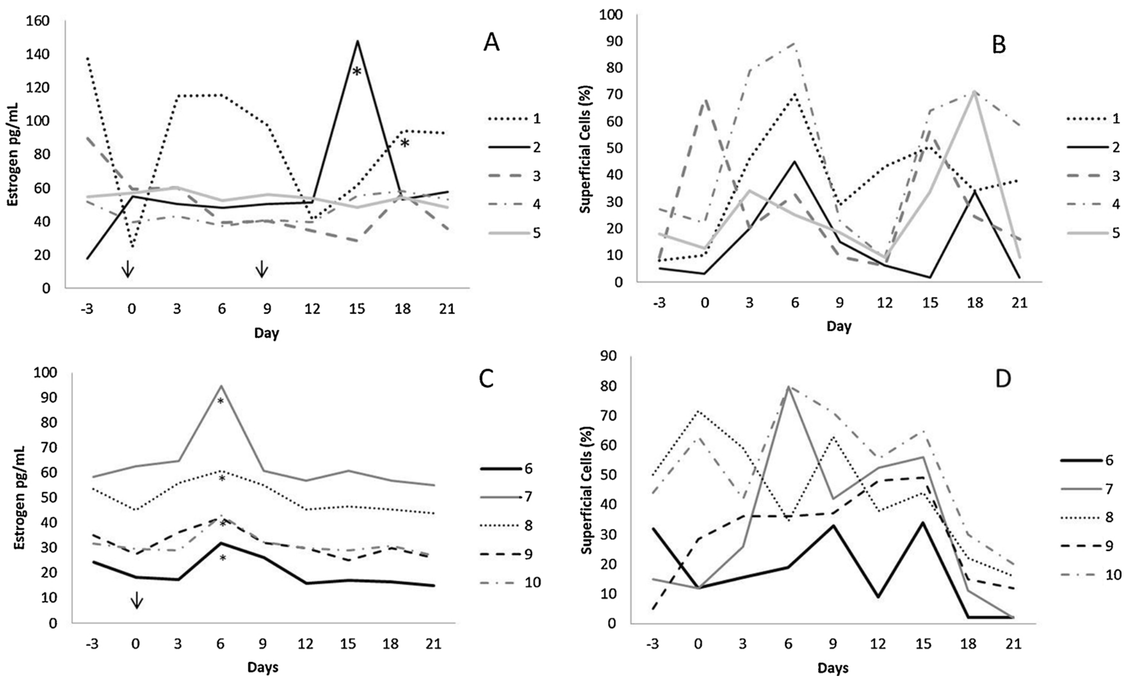

Fig. 1. Longitudinal evaluation of collared peccary females ($n = 10$) subjected to different treatments for estrous synchronization; A and B – 17β-estradiol profile (pg/mL) and proportion of superficial cells in vaginal cytology evaluations of females treated with double injection of cloprostenol (at day 0 and 9 as indicated by arrows); C and D – estrogen profile (pg/mL) and proportion of superficial cells with vaginal cytology of females treated with single injection of eCG/hCG (at day 0 as indicated by arrow); *Asterisk indicates the occurrence of 17β-estradiol peaks.

Table 2
Progesterone values at the commencement of the study and estrogen concentration at the onset of estrus signs, proportions of cell types found in vaginal cytology, total number of follicles per ovary and average diameter of the follicles as detected by ovarian ultrasonography conducted between 6 and 9 days after the second cloprostenol administration or at six days after the eCG/hCG administration (n = 5) in female collared peccary*.

	Female #	Progesterone value (ng/mL)	Estrus signs	17β-estradiol Peak (pg/ml)	Vaginal Cytology (%)			Ovarian ultrasonography		
					Parabasal	Intermediate	Superficial	Diameter of antral follicles (cm)	Nº of follicles on left ovary	Nº of follicles on right ovary
Cloprostenol	1	64.7	+	94.2	14	6	49	0.6 ± 0.2	2	3
	2	118.9	+	147.8	29	35.5	33.5	0.3 ± 0.1	2	2
	3	193.4	+	56.8	38	28	34	0.8 ± 0.3	1	2
	4**	123.4	–	–	–	–	–	–	–	–
	5	73.9	+	54.3	60	30	10	0.4 ± 0.1	3	1
	Mean ± SEM	114.9 ± 22.9		89.5 ± 21.2	32.3 ± 9.6	24.9 ± 6.5	31.6 ± 8.1	0.5 ± 0.2	2 ± 0.4	2 ± 0.4
Gonadotropins	6	56.3	+	32.0	42	39	19	0.3 ± 0.1	2	1
	7	92.3	+	94.9	9.2	11	79.8	0.6 ± 0.2	4	1
	8	72.9	+	60.2	19.5	46	34.5	0.7 ± 0.2	1	3
	9	90.6	+	42.1	41.3	22.7	36	–	0	0
	10	56.6	+	43.0	3	17	80	0.3 ± 0.1	2	0
	Mean ± SEM	73.8 ± 7.8		54.4 ± 22.2	23.0 ± 16.1	27.1 ± 13.3	49.9 ± 25.2	0.5 ± 0.2	2.3 ± 0.6	1.3 ± 0.6

* No significant difference related to any characteristic (estrus occurrence, 17β-estradiol levels, cell proportions, diameter or number of antral) was detected between treatment groups ($P > 0.05$).
** Female nº 4 did not respond to the cloprostenol treatment.

cloprostenol and 100% for gonadotropins combination) was similar ($P > 0.05$).

Three females (Nº 2, 3, and 5; Table 2) treated with cloprostenol expressed symptoms of estrus 9 days after the second hormone administration, and one female (Nº 1) had symptoms of estrus precociously, after 6 days. There were the greatest 17β-estradiol concentrations (Fig. 1A) concomitant with symptoms of estrous manifestation, including open, swollen, and hyperemic vulva, vaginal mucus, and easy penetration of swabs (Fig. 2). For vaginal cytology (Table 2), only one individual (Nº 1) had a predominance of superficial cells during estrus (Fig. 1B), but the presence of red blood cells was detected in three individuals (Nº 1, 2 and 5). Presence of large antral follicles was detected using ultrasonography in all estrous females.

All five females treated with eCG/hCG presented estrous symptoms 6 days after hormone treatment, concomitant with the increase in 17β-estradiol concentrations (Fig. 1C; Table 2). Only two females (Nº 7 and 10) had a predominance of superficial cells during estrus, as detected using vaginal cytology (Table 2; Fig. 1D). Large antral follicles were detected using ultrasonography in all individuals manifesting estrus, except one (Nº 9; Table 2).

Males that were placed with both groups of females detected symptoms of estrous manifestation as indicated by expressed sexual behaviors associated with mating, including curled lips while expressing the Flehmen reflex. Males also expressed territory demarcation, consisting of scrubbing the dorsal gland throughout the enclosure. Females in estrus allowed males to approach and natural mating occurred; however, there were no resulting parturitions of any female during the following 150 days.

3.2. Experiment 2 – Artificial insemination study

On the day of gonadotropin administration, seven females were in luteal phase and three females were in follicular phase of the estrous cycle (Table 3). All females expressed symptoms of estrus on day 6 after hormone administration, when the first AI was conducted, concomitant with increasing 17β-estradiol concentrations (221.5 ± 66.9 pg/mL). At day 8 post-treatment, when the second AI was performed, females continued to manifest symptoms of estrus, and had greater than basal 17β-estradiol concentrations (185.0 ± 67.1 pg/mL) as well as blood discharge occurring in four of ten females.

The results from ultrasonic evaluations indicated there were no experimental females that became pregnant as a result of AI. At 30 days after AI, however, all females had sustained concentrations of progesterone that were greater than basal with these concentrations being sustained in seven females to day 60 (Table 3). There was no symptoms of estrus detected in these seven females during the experiment, whereas with the other three females there was a resumption of estrous cycles. Furthermore, the two females that had the greatest progesterone values (Female 1–98.1 ng/ml; Female 8–93.7 ng/ml) at 60 days after AI had the largest uterine volume with an obvious presence of anechoic liquid (Fig. 3), with thickening walls (0.9 cm each wall) in comparison to day 0 when the uterine wall had had a thickness of 0.5 cm without a visible lumen.

3.3. Experiment 3 – Macroscopic examination of ovaries

Six days after gonadotropin administrations, at the time of necropsy of two females there were symptoms of estrus confirmed by the occurrence of ovarian stimulation (Fig. 4). In Female 11, three hemorrhagic bodies and 88 antral follicles were identified, whereas Female 12 had eight hemorrhagic bodies and 25 antral follicles (Table 4).

Fig. 2. Estrous symptoms of the external genitalia of collared peccaries: (A) Vulva of a female in the luteal phase of the estrous cycle at the beginning of the experiment; (B) Hyperemic and swollen (C) and open vulva of a female in estrus; (D) Presence of blood on the swab used to ascertain vaginal cytology of a female in estrus.

Table 3
Progesterone blood values (ng/mL) for collared peccary females (n = 10) at the day of gonadotropins administration and at 30 and 60 days after artificial insemination (AI).

Females #	Progesterone values (ng/mL)		
	On the day of drugs administration	30 days after AI	60 days after AI
1	2.5*	21	98.1
2	19.6	209.5	40.5
3	57.52	179	**
4	63.2	312	***
5	55.9	179	32.7
6	1.5*	55.9	38.8
7	92.3	156	50.10
8	40.8	100.7	93.7
9	90.6	298	***
10	1.2*	180	***

* Females 1, 6 and 10 were in the follicular phase at the day of drugs administration, while others were in luteal phase.
** Not enough volume for hormone analysis.
*** Females had returned to natural cycle.

4. Discussion

In the present study, there were new data regarding the use of a gonadotropin combination for estrous synchronization in the collared peccary. The eCG/hCG combination was as effective for estrous synchronization in peccaries (100%) as in pigs with 90% of pigs expressing a synchronous time of estrus (Knox et al., 2000). These gonadotropins have already been extensively used in pigs, in which eCG is known to stimulate ovarian follicular development and hCG to induce ovulation (Ziecik et al., 2017). The ovulation rate, however, is generally associated with the dose of eCG administered (Karalus et al., 1990), and the establishment of adequate doses or even the association of gonadotropins with other hormones, such as prostaglandins (De Rensis et al., 2012) are necessary for

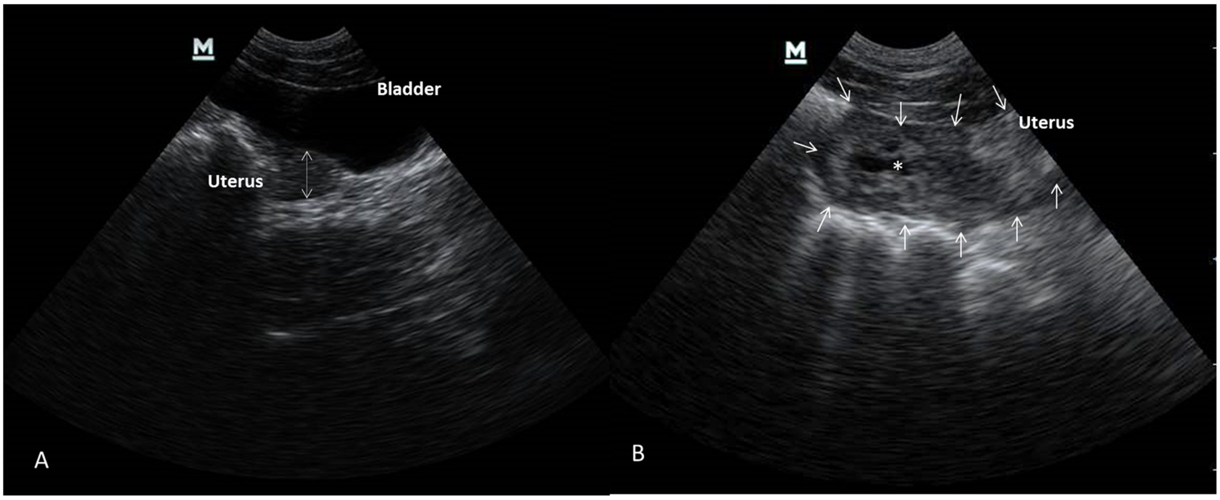

Fig. 3. Sonogram of two collared peccary females (nº 1 and 8) with greater than basal progesterone for 60 days after artificial insemination indicating the increased uterine volume with thickened walls (arrows) and containing luminal anechogenic liquid (*).

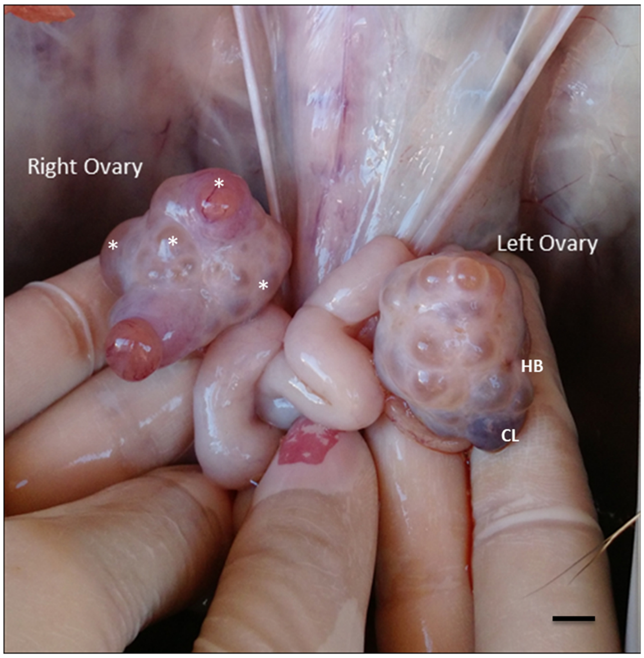

Fig. 4. Left and right ovaries from a female collared peccary 6 days after administration of eCG/hCG; Note the presence of various follicles (*), a hemorrhagic body (arrow) and a corpus luteum (arrow head) Scale bar 1:0.5 cm.

Table 4
Number and classification (per diameter) of antral follicles and hemorrhagic bodies in the ovaries of female collared peccaries (*Pecari tajacu*) six days after injection of gonadotropins combination.

Ovarian structures	Female 11		Female 12	
	Right Ovary	Left Ovary	Right Ovary	Left Ovary
Follicles				
Small (< 0.3 cm)	5	13	1	2
Medium (0.3–0.5 cm)	7	20	5	4
Large (> 0.5 cm)	10	13	7	6
Total	22	46	13	12
Hemorrhagic bodies	1	2	5	3

effectiveness in production of piglets as a result of AI.

In the present study, the gonadotropin combination was effective for estrous synchronization and ovarian stimulation in collared peccaries. This protocol was recently used to obtain many viable oocytes to be used for in vitro maturation/fertilization and embryo production in this species (Borges et al., 2019). Additionally, ovaries from collared peccary females stimulated with gonadotropins were useful to study some aspects of folliculogenesis through the analysis of various gene expression, such as those for follicle stimulating (FSH) and luteinizing (LH) hormone receptors, in follicles from the reserve pool to the antral phase (Freitas et al., 2018a, 2018b).

Results of recent studies indicated there were effective actions of a gonadotropin combination when it was used in gilts or weaning sows (Estienne and Crawford, 2015). Knox (1999) also reported the use of the same hormones used in the present study for estrous induction in sows at varied stages of estrous cycle but with variable results. Interestingly, collared peccary females seem to respond to the hormones, both when these are administered during the luteal or follicular phases, as observed in Experiment 2 of the present study. Collared peccaries had a synchronized estrus 6 days after eCG/hCG injection with a concomitant increase in estrogen concentration and presence of large antral follicles, reducing the probability of an incorrect estrous detections similar to what occurred in pigs (Kaneko et al., 2013). Thus, gonadotropin treatment may improve the reproductive management and production (Estienne and Crawford, 2015). Furthermore, the use of eCG/hCG combination as a single dose reduces stress because there is requirement of only a single animal restraint session. Additionally, estrogen concentrations of females treated with either prostaglandin or gonadotropins were similar to those reported for females during a naturally occurring estrus (Maia et al., 2014a).

Besides eCG/hCG treatment, the use of prostaglandins was also effective (80%) for estrous induction in collared peccaries; however, did not have an adequate estrous synchronization among females because of expression of estrus at 6 or 9 days after hormone administration. Delayed responses to estrous synchronization (9.5 ± 0.5 days) using a smaller dosage of prostaglandin (60 μg) was previously observed in the same species (Maia et al., 2014b), but there was verification that an increase in the prostaglandin dosage did not positively affect the estrus occurrence in this species. Additionally, the luteal phase in the collared peccary lasts 15 days on average (Maia et al., 2014a), similar to that of gilts (13–15 days; La Voie, 2017). A second prostaglandin administration, therefore, is recommended to promote the lysis of corpus lutea that were not developed at the time of the initial hormone administration (Maia et al., 2014b). Even with the use of two consecutive prostaglandin injections, these results indicate that collared peccaries have a marked variation in the response to the prostaglandin treatment similar to that reported for swine (De Rensis et al., 2012).

Treatment for estrous synchronization using eCG/hCG was sufficiently effective in the induction of ovulations and the formation of corpora lutea, which are essential for establishing early pregnancy. At 60 days after AI, there had been no observations of estrus in seven females that had luteal functionality in which there was imposing of the estrous synchronization treatment regimen. Greater than basal progesterone concentrations could be related to uterine modifications such as those evidenced in two females detected by ultrasonography. These findings indicate the presence of functional corpora lutea at 60 days after AI after a time interval that is significantly greater than that of 15 days reported for the luteal phase in the natural estrous cycle of this species (Maia et al., 2014a). Because it has been reported that administration of gonadotropins (1500 IU eCG and 1000 IU hCG) could result in decreased prostaglandin synthesis in the uterus of swine, causing disruption of the oviductal environment (Małysz-Cymborska and Andronowska, 2015), further experimental studies of dose-response for gonadotropins should be conducted to refine estrous synchronization in collared peccaries.

Observations resulting from dissection of collared peccary ovaries indicated there was variation in the individual response of females to the gonadotropin treatment, because hormonal treatments resulted in an excessive stimulation of antral follicles (88 antral follicles) in one female, but not in the other (25 antral follicles). The number of hemorrhagic bodies detected in the second female was consistent with what would occur as a result of natural ovulation, 2.3 ± 0.6 follicles, reported for this species (Mayor et al., 2006). In addition, the presence of a corpus luteum in the ovary of one female indicated there was a need for an associated prostaglandin administration with the gonadotropin protocol to promote the luteolysis in females treated during the luteal phase, which which is consistent with what has been reported for fertile estrous induction in swine (De Rensis et al., 2012).

During natural estrus of peccaries, there was a larger diameter of pre-ovulatory follicles reported as 0.9 ± 0.5 cm than that of ovulatory follicles in the present study (Mayor et al., 2006). In the present study, both ultrasonic examinations and necropsies indicated the occurrence of ovarian follicles larger than 0.5 cm concomitant with an increased plasma 17β-estradiol concentration; thus, reinforcing that there was the occurrence of estrus in treated females, as observed in pigs after prostaglandin or gonadotropin administrations (Kiewisz et al., 2011).

In summary, there was demonstration of the effectiveness of the single administration of eCG/hCG to properly synchronize time of estrus among collared peccary females. These encouraging results are important and valuable information that can be used for improving the use of assisted reproductive techniques for the management of collared peccary populations.

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