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Laparoscopic ovum pick-up in spotted paca (*Cuniculus pacas*)

[*Aspiração folicular por laparoscopia em paca (*Cuniculus pacas*)*]

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ABSTRACT

The aim of this work is study the laparoscopic ovum pick-up (LapOPU) technique in spotted paca, describing surgery details, complications and oocyte recovery rate. Nine healthy adult non-pregnant captive females were used, in a total of 39 procedures. When the surgical plane of anaesthesia was achieved, the females were positioned at 20º Trendelenburg. Three 6mm trocars were placed on right and left inguinal and hypogastric regions. Abdomen was inflated with CO_2 and the intra-abdominal pressure was stablished in 10mmHg. Follicular punctures were performed moving the ovaries with atraumatic forceps. For punctures, an 18-gauge 3.5 inch long needle attached to a vacuum system with pressure not exceeding 65mmHg was used. Oocytes were recovered into 50mL centrifuge tubes with media composed of PBS supplemented with 10 IU/mL of heparin and kept at 36°C. R Software was used for statistical analysis. Data normality distribution (Shapiro test) and variances homoscedasticity (Bartlett test) were tested and descriptive statistics (mean±SD) was used to present the results. It was only possible to perform LapOPU in 30 of 39 laparoscopies (76.92%). The surgical total time was 37.34 ± 18.53 minutes. The total number of visualized follicles, aspirated follicles, and retrieved oocytes were 502, 415, and 155, respectively. And the same parameters per animal were: 14.34 ± 12.23, 11.86 ± 10.03, and 4.43 ± 4.69 respectively. Oocyte recovery rate was 32.56 ± 27.32%. In conclusion, caudal positioning of portals with slight triangulation allows good viewing of the abdominal cavity and eases the manipulation of the ovaries. Thus this described LapOPU technique is feasible in spotted paca and easy to perform.

Keywords: follicular aspiration, laparoscopy, oocyte, rodent, wild animals

RESUMO

Objetiva-se, com este trabalho, estudar a técnica de aspiração folicular por videolaparoscopia (LapOPU) em pacas, descrevendo detalhes do procedimento cirúrgico, complicações e taxa de recuperação oocitária. Para isso, foram utilizadas nove fêmeas, saudáveis, adultas, não gestantes e mantidas em cativeiro, totalizando 39 procedimentos. Quandoo plano anestésico cirúrgico foi alcançado, as fêmeas foram posicionadas em Trendelenburg com 20º de angulação. Três trocáteres foram colocados nas regiões inguinais direita e esquerda e hipogástrica. O Abdômen foi insuflado com CO_2, e a pressão intra-abdominal foi mantida em 10mmHg. Punções foliculares foram realizadas manipulando-se os ovários com pinças atraumáticas. Para aspirações foliculares, usou-se agulha de 18G com bisel curto acoplado ao sistema de vácuo com pressão não excedendo 65mmHg. Oócitos foram recuperados em tubos de centrifugação de 50mL contendo meio composto de PBS suplementado com 10UI/mL de heparina e mantidos a 36ºC. Usou-se software *R para análise estatística.*
Testaram-se a distribuição normal dos dados (teste de Shapiro) e a homocedasticidade das variâncias (teste de Barlett) e se usaram estatísticas descritivas (média±DP) para apresentar os resultados. Das 39 videolaparoscopias, só foi possível realizar LapOPUem 30 delas (76,92%). O tempo cirúrgico total das LapOPU foi de 37,34 ± 18,53 minutos. Os números totais de folículos visualizados, folículos aspirados e oócitos recuperados foram: 502, 415 e 155, respectivamente. E os mesmos parâmetros por animal foram:

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14,34 ± 12,23, 11,86 ± 10,03 e 4,43 ± 4,69, respectivamente. A taxa de recuperação foi de 32,56 ± 27,32%. Assim, conclui-se que o posicionamento caudal de portais, com ligeira triangulação, permite uma boa visualização da cavidade abdominal e facilita a manipulação dos ovários, sendo essa técnica de LapOPU viável em pacas e de fácil execução.

Palavras-chave: aspiração folicular, laparoscopia, oócito, roedor, animais selvagens

INTRODUCTION

Spotted paca is the second largest neotropical rodent (Matamoros 1982). According to the International Union for Conservation of Nature, this species is not in danger of extinction (IUCN 2015), but overall its numbers have been much decreased because of hunting and habitat destruction (Queirolo *et al*., 2008). It is easily bred and raised in farms (Guimarães *et al*., 2008; Hosken and Silveira 2001), thus the commercial breeding of this species appears as an alternative of conservation, by increasing their population stock, and also promoting a reduction in hunting and trafficking pressure, and to fill the growing demand for exotic meats in large urban centers (Lourenço *et al*. 2008).

This growing demand favors the development of scientific researches involving biotechnology on reproduction, adapting techniques developed for domestic animals, on the conservation of threatened wild species or to improve zootechnical production of those kept in commercial farms (Pizzi *et al*., 2013).

Laparoscopic ovum pick-up (LapOPU) used for in vitro production (IVP) is one of the leading reproductive biotechniques in assisted reproduction, highlighted for being less invasive, provide a quicker recovery and the possibility of being held several times in the same female as already described in some domestic species (Teixeira *et al*., 2011; Cordeiro *et al*., 2014; Padilha *et al*., 2014; Teixeira *et al*., 2015).

Due to the absence of description of this technique in *Cuniculus paca*, the aim of this study was to describe LapOPU in this species, reporting operative details, complications, and oocyte recovery rates.

MATERIAL E METHODS

The present study was conducted following the approval of the Animal Ethics and Welfare Committee of the College of Agricultural and Veterinary Science of the Universidade Estadual Paulista (protocol n. 027420-11). The ethical principles of the European Commission for experiments involving animals (Directive 83/609EEC) were also followed.

Nine adult female spotted pacas (n = 9), older than seven months, weighing an average of 9kg, non-pregnant and healthy were used in this work. All of them came from the Wild Animal Section of the Department of Animal Science FCAV / UNESP (registration at IBAMA - 482508). Two pacas were submitted to six laparoscopies, one paca to five, five pacas to four, and one paca to two laparoscopies, totaling 39 procedures.

For performing the LapOPU, animals received as pre-anesthetic medication (PAM), 25mg / kg of ketamine IM (Ketalar®, Pfizer, Brazil) and 0.5mg/kg of midazolam IM (Dormonid®, Roche, Brazil). Subsequently, all animals were induced and maintained on isoflurane (Forane®, Abbott, Argentina) provided in the open system by face mask, diluted in 100% O2. Intraoperative IM tramadol 5 mg/kg (Tramadon®, Cristalia Laboratories, Brazil) or methadone 0.5 mg/kg (Mytedom®, Cristalia Laboratories, Brazil) administered for pain control.

Then surgical area, on the abdomen, was clipped and prepared using routine aseptic technique with chlorhexidine gluconate 2%. All procedures were carried out by skilled staff on reproductive laparoscopic procedures, however, this technique has never been achieved with this species.

When the surgical plane of anaesthesia was achieved, females were positioned in Trendelenburg 20º. At the sites of incisions, anesthetic buttons were made with 1mL of lidocaine 2% (Lidovet®, Bravet, Brazil). Then, with a scalpel, three skin incisions were made for establishing the three laparoscopic portals, with 5-mm cannulas.

Initially, in the first three procedures, the best portal arrangement was studied. The first trocar-cannula containing insufflation valve was inserted through the Hasson technique, on the

right inguinal region. After insertion, the trocar has been removed, remaining in place only the cannula, where it was connected to a silicone hose through the insufflation valve, coming from the electronic insufflator. Then the abdomen was insufflated by infusing carbon dioxide (CO_2) and intra-abdominal pressure (IAP) set to 10mmHg, using a 2L/min insufflation rate.

When established a pneumoperitoneum, a 5mm, 0-degree laparoscope connected to a video camera and a fiber optic cable was inserted through the cannula for a brief abdominal exploration. Subsequently, it made up the video-assisted introduction of other two trocars. The second trocar was inserted on the hypogastric region, on the midline, and the third trocar was introduced symmetrically to the first one, on the left inguinal region, thus maintaining a light triangulation (Figure 1A).

After the establishment of laparoscopic portals, the laparoscope was transferred to the second cannula and through the other portals 5-mm atraumatic grasping forceps were inserted (Babcock®, Bhio Supply, Brazil) for manipulation of the uterus, fallopian tubes, ovarian bursa, and ovaries.

Using the Babcock forceps, the uterus was manipulated in search of the ovaries, localization facilitated by a partial lateralization handling of the animal. In right lateral decubitus for the manipulation of the left ovary and left lateral decubitus, for the manipulating of the right one (Figure 1B)

At the time of grasping the ovaries it was possible to count the number of follicles. Each ovary was held by only one of the forceps, and brought close to the abdominal wall. Moreover, using the Babcock, it was possible to move it, which facilitated the follicular puncture. For a better suction of all follicle contents, needles were rotated or moved carefully when inside of them (Figure 1C).

Figure 1. Follicular aspiration by laparoscopy in *Cuniculus paca*. In (A) and (B) patient positioning and external view of the arrangement of laparoscopic portals in the caudal-cranial direction with the animal in supine position, first portal with inflation valve (red "x"), second portal with rigid endoscope inserted (blue circle), third portal (green asterisk). In (B) use of the lateralization handling and puncture (purple triangle). In (C), the insight follicular puncture needle inserted into the follicle (1), pressure clamp (2) holding the uterine horn (3) and ovarian (4).

The vacuum system used, a simple lumen type (same internal diameter), was composed of a 18G needle with short bevel, connected to a 50cm length cannula, connected to a silicone cork (Handle Cook®, Brazil) which were connected to the collection tube (50mL). The vacuum was produced by a vacuum pump (BV 003d®, WTA, Brazil) connected to silicone tubing also connected to the collection tube. The vacuum pressure was set to 65mmHg.

Prior to the puncture of the oocytes, the rinsing of the system was performed using medium harvest, leaving approximately 2mL of medium for receiving oocytes after this procedure. At the end of aspiration, the ovaries were washed with 2% lidocaine (7mg/kg) diluted in 20ml of 0.9% NaCl saline solution, removing clots formed on the surface, in order to minimizeadhesion formation. Then, forceps and cannulas were removed, while removing CO_2. Skin suture was performed using horizontal "U-shaped" suture pattern and poliglecaprone 25 sutures (Caprofyl® 2-0, Ethicon Inc, USA) were applied. The total length of surgery, and the first incision to the suture were recorded in minutes.

The surgical wounds were treated with chlorhexidine gluconate 2% followed by the application of repellent ointment (Ointment Pearson®, Eurofarma, Brazil) around each one.

Following surgery, animals were placed in a clean and quiet environment and observed until the return of anesthesia and acquiring standing position. After that, all returned to their enclosures.

During the early post-operative period, all animals received 0.3mg/kg IM of meloxicam (IM, Maxicam®, Ourofino, Brazil) SID for 3 days and a single dose of a combination of penicillin (G potassium, procaine and benzathine 15,000IU/kg) with streptomycin (10mg/kg) (IM, Penfort PPU®, Ourofino, Brazil).

Oocytes were recovered in the harvest medium consisting of PBS supplemented with 10IU/mL heparin, heated in a water bath at 36°C and processed in the laboratory.

R Software (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. Data normality distribution (Shapiro test) and variances homoscedasticity (Bartlett test) were tested and descriptive statistics (mean±SD) was used to present the results.

RESULTS

The anesthetic protocol was safe and effective, keeping the animals in an appropriate anesthetic plan, muscle relaxation, and analgesia. All females showed excellent anesthetic recovery and quick healing of surgical wounds.

In 39 laparoscopies, it was possible perform LapOPU in 30 of them (76.92%). The total surgical time of LapOPU was 37.34±18.53 minutes. The times in minutes for each phase of laparoscopies were the incision to insert the first trocar 2.44±1.39; insufflation 2.55±3.57; pneumoperitoneum property at the entrance of the second trocar 2.60±1.57; the second input of the third insertion trocar 1.24±0.67; the third trocar entry to the beginning of the first manipulation ovarian 4.21±2.65; manipulating the first ovary 15.35±7.40; Manipulation of the second ovary 13.33±8.29; deflation until skin suture 5.71±2.75.

The total number of visualized follicles, aspirated follicles, and oocytes retrieved were: 502, 415 and 155, respectively. And the same parameters per animal were: 14.34±12.23, 11.86±4.43 and 10.03±4.69 respectively.

Prior to LapOPUs, the animals were not submitted to fasting, and there were no complications related to the gastrointestinal tract. The IAP 10mmHg with blowing speed of 2L/min, together with the positioning of laparoscopic portals were efficient and promoted excellent visualization of the abdominal cavity, which was transmitted to the monitor with 10x magnification.

During the first three procedures, trocars were introduced into the cranial abdominal region (right and left hypochondriac and epigastric), but we found that it was more feasible the positioning of portals with light triangulation directing the instrument in the caudal direction, added to partial handling lateralization of the forelimbs, as described, making the viewing and manipulation of the ovaries easier.

The vacuum system used for ovum pick up, which allowed a maximum pressure of a 65mmHg, demonstrated to be efficient for the recovery of viable oocytes in spotted pacas obtaining an oocyte recovery rate of 32.56±27.32%. However, in one of the procedures it was not possible to perform follicular aspiration due to the malfunction of the vacuum pump. The problem was solved for the other procedures.

In some procedures minor bleeding was observed at the time of the punctures. Adhesions of ovary and adjacent tissues to the peritoneum and abdominal wall were observed in 5 of the 9 animals (55.56%) , but only four procedures from 39 (10.26%) were unable to perform oocyte recovery due to such complications (Fig. 2A). One patient of 9 (11.11%) was submitted to six procedures, showing no complications and 3 of 9 animals (33.33%) submitted to four procedures, also without such complications.

Regarding other events, one hemorrhage was observed during attempts at adhesiolysis, which prevented the oocyte recovery, but without conversion or more invasive hemostasis needed, only requiring washing and aspiration, performed

by videosurgery (Figure 2B). Also, the urinary bladder was perforated at the first entrance portal during one procedure, requiring conversion to peritoneal lavage and bladder suture (Figure 2C). However, the same patient was used in three more procedures without any adhesion formation. After this complication, bladder catheterizations were performed in all females, so they went into anesthesia, just before the introduction of the first trocar.

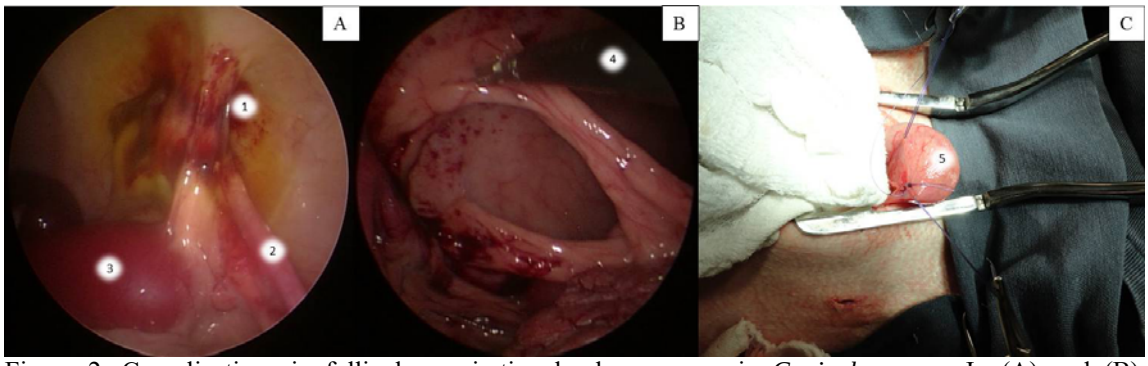

Figure 2. Complications in follicular aspiration by laparoscopy in *Cuniculus paca*. In (A) and (B) inside view of adhesion (1) reproductive tract (3) in the right kidney (2) and peritoneum, and adhesiolysis using Babcock clamp (4). In (C) external view of cistorrafia after accidental perforation of the urinary bladder (5).

DISCUSSION

The anesthetic protocol used in this experiment was shown to be as safe and efficient as similar to previously described in this species (Vilani *et al*. 2004).

The spotted paca has anatomical positioning of the ovaries and uterus similar to dogs (Feliciano *et al*. 2014), thus portal positioning was similar to the three portals ovariohysterectomy technique used for bitches (Brun *et al*. 2000), making the viewing and manipulation of the ovaries easy.

Not performing fasting before each procedure is a practice used in rodents, as these animals do not regurgitate, moreover have high metabolic rates and fasting could deplete energy reserves quickly (Bernal *et al*., 2009).

Surgical time was similar to those achieved by Teixeira *et al*. (2011) with 26.75±9.6 min. working with sheep and Cordeiro *et al*. (2014), lasting 35 min. with goats. Times of manipulation and ovarian punctures proved to be the most complex steps in LapOPU procedure, possibly due to the number and size of follicles, these results corroborate those found by Teixeira *et al*. (2015), in addition to the lateralization of animals facilitating access and manipulation of both ovaries, as shown by Silva *et al*. (2015).

Several authors reported oocyte recovery rates ranging from 16 to 98% (Alberio *et al*., 2002; Gibbons *et al*., 2007; Teixeira *et al*., 2011; Cordeiro *et al*., 2014) in small ruminants. Due to the shortage of LapOPU data in rodents, it can be assumed that the recovery rate achieved in our study (32.56±27.32%) is in accordance with what Rodríguez *et al*. (2006) considered normal (from 32 to 90%).

The used pressure of 65mmHg on the vacuum pump in this study was slightly higher than that Morton *et al*. (2008) considered bringing better results (50mmHg) in follicular aspirations in sheep. But the use of 18G needles, goes according to Rodríguez *et al*. (2006), which obtained excellent oocyte recovery rates in sheep using the same gauge needles or slightly thinner (20G). Even though within the normal range, we believe that the caliber of the used needle could have caused ovarian bleeding, which is reported as one of the complications of follicular aspiration (Sarhan and Muasher 2007).

As a consequence of the bleeding there were peritoneal adhesions, which sometimes difficult the viewing and aspiration of follicles,

disfavoring our recovery rate. But over the course of the experiment, to try to prevent adhesions, the abdominal cavity was washed using saline solution (NaCl 0.9%) added to lidocaine 2%, as this local anesthetic has been awarded as one of the drugs that act on prevention of adhesions, for its possible anti-inflammatory effect (Ward and Panitch 2011). However, we could not avoid them. Yuzbasioglu *et al*. (2008) reported that in the induced peritonitis in rats, the lidocaine associated to prilocaine was more effective in preventing intra-abdominal adhesions than lidocaine alone. Cordeiro *et al*. (2014), working with goats, observed that 15% had adhesions, even when washing the cavity with diluted heparin solution in PBS. Teixeira *et al*. (2011) reported no such deleterious consequences, even washing the cavity with only NaCl 0.9%, and added that this may be due to the careful manipulation of the genital tract, and removing clots. More adhesion prevention studies are still needed in spotted pacas.

The bladder perforation is an injury already reported in access by the Hasson technique in other species, being described as one of the conversion conditions (Armenakas *et al*., 2004; Pillet *et al*., 2009; Ferreira *et al*., 2013). However, the correction was uncomplicated (Waldron 2003). A month after the incident, the animal returned to the experimental group, without damage to the reproductive tract.

According to Tams and Rawlings (2011), such accidents are rare. And this has also rarely occurred in our routine. Duarte *et al*. (2009) reports that these types of eventualities could be avoided if we use the Veress needle inflating the abdominal cavity, before the entry of the first trocar.

CONCLUSION

Caudal positioning of portals with slight triangulation allows good viewing of the abdominal cavity and eases the manipulation of the ovaries. Even with existing possibility of complications, these were not as significant, and this technique demonstrated to be feasible in this species, with the advantage of being of rapid execution and promoting good recovery of animals after the procedure, especially due to its minimally invasive nature.

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