Ovum pick up (LOPU) in Santa Ines sheep: learning curve and technical details

Aspiração folicular ovariana em ovelhas da raça Santa Inês: curva do aprendizado e detalhes técnicos

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Abstract

The aim of the current study was to assess the efficacy, the learning curve and associated technical difficulties of laparoscopic ovum pick-up (LOPU) in sheep. Ten ovum pick-up sessions were performed in six Santa Ines sheep during a cycle of 7-day intervals, totaling 60 laparoscopic procedures. The sheep were managed for synchronization of the estrous cycle. The laparoscopic approach was carried out using three ports. The number of follicles viewed, aspirated follicles and oocytes recovered were 13.32 ± 2.8, 11.37 ± 2.8 and 6.36 ± 2.0, respectively. Total operation time was used to assess the learning curve among 10 laparoscopic sessions. Mean surgical time was relatively short (26.75 ± 9.6 minutes). Surgical time decreased from the first session on ($P < 0.05$). LOPU was demonstrated to be a procedure with little intercurrence and with a short learning curve. Others studies regarding this technique should be performed to minimize possible complications and enabling its use in the field.

Keywords: Ovum pick-up. Sheep. Intraoperative period. Laparoscopy.

Resumo

O objetivo do presente estudo foi avaliar a eficácia, a curva de aprendizado e a detecção de dificuldades técnicas na videolaparoscopia para a aspiração folicular (LOPU) em ovinos. Foram realizadas dez sessões de LOPU em seis ovelhas Santa Inês durante um ciclo de sete dias de intervalo, totalizando 60 procedimentos laparoscópicos. As ovelhas foram submetidas à sincronização do ciclo estral. A abordagem laparoscópica foi realizada utilizando-se três portas. O número de folículos observados, aspirados e oócitos recuperados foi de 13,32 ± 2,8, 11,37 ± 2,8 e 6,36 ± 2,0, respectivamente. O tempo total dos procedimentos foi cronometrado para avaliar a curva de aprendizagem entre dez sessões laparoscópicas. O tempo cirúrgico gasto foi de 26,75 ± 9,6 minutos, reduzindo-se a partir da primeira sessão para a última (P < 0,05). A LOPU demonstrou ser um procedimento de poucas intercorrências, com uma curta curva de aprendizado; recomenda-se outros estudos relativos à técnica visando a reduzir possíveis intercorrências e disponibilizando o uso a campo.

***Palavras-chave**: Aspiração Folicular. Ovelhas. Transoperatório. Laparoscopia.*

Introdution

Ovum pick-up plays in domestic animals a crucial role in vitro production of embryos. The oocytes can be harvested from ovaries obtained either from slaughterhouses, surgical resection of the ovaries or *in vivo* by ovum pick-up (OPU) using transvaginal ultrasound guidance, laparotomy or laparoscopic techniques (WANI, 2002).

Oocyte recovery using transvaginal ultrasound-guided OPU is widely performed in bovines. Such technique presents several limitations in the small ruminant practice. Besides more traumatic than laparotomy or laparoscopic approach, transvaginal ultrasound-guided OPU is technically challenging even for skilled practitioners. On the other hand, laparotomic accessed OPU inevitably implies in moderate or severe surgical trauma, frequently leading to complications such as surgical wound infection or dehiscence, pain, postoperative adhesion formation and decreased productive and reproductive performance (BASSO et al., 2008). Such disadvantages are deleterious in medium and long term, and compromise the productive longevity of females of high genetic value (FREITAS; SIMPLÍCIO, 2002).

Laparoscopic access for recovery of oocytes is a minimally invasive procedure, which allows faster surgical recovery and multiple OPU in several different moments in the same female. Such technique has been used in small ruminants in order to obtain oocytes *in vivo* for basic research and for *in vitro* production of embryos (BALDASSARRE, 2012; CORDEIRO, 2006). Despite of the benefits of the minimally invasive surgery, the learning of laparoscopic approach is one of the major challenges for the popularization of the ovum pick-up technique in small ruminant practice. Although laparoscopic OPU has been widely used in other studies for oocytes achievement in sheep (BALDASSARE; KARATZAS, 2004; BASSO et al., 2008; WIECZOREK et al., 2010), no studies focusing on the learning curve, i.e., on the number of procedures required to reach optimal operation time and minimized intraoperative complication rate by non-proficient surgeons, have been performed so far.

Therefore, the aim of the current study was to evaluate the learning curve of the laparoscopic ovum pick-up (LOPU) technique in Santa Inês sheep with emphasis on the intraoperative steps, operation time and possible surgical complications.

Material and methods

The present study was conducted following the approval of the Animal Ethics and Welfare Committee of the School of Agrarian and Veterinary Sciences of the São Paulo State University (protocol n. 025988-08). The procedures were performed in six adult Santa Inês ewes with a mean body condition score of 3.0 (1 = thin-5 = obese) (JEFFERIES, 1961). The animals were considered healthy following clinical and hematologic examination and ultrasound evaluation of the reproductive tract. The surgical procedures were assessed

on six sheep during each of 10 subsequent LOPU sessions, totaling 60 procedures.

Before the beginning of the study, the ewes were submitted to synchronization of estrus using 60 mg of intravaginal medroxyprogesterone acetate (MAP, Progespon®, Schering-Plough, Brazil) for six days, intramuscular administration of 37.5 µg of D-cloprostenol (Sincrocio®, Ourofino S.A, Brazil) and 300 IU of eCG (Novormon®, Schering-Plough, Brazil) on the fifth day of the protocol. The ovarian stimulation was performed 12 hours after detection of estrus (confirmed with teaser) and consisted of administration of 80 mg of FSHp (Folltropin®, Schering-Plough, Brazil) and 300 IU of eCG, and after 36 hours the ovum pick-up procedures were performed by laparoscopy. For the subsequent aspirations, only the ovary stimulation was performed.

The animals were fasted for 36 hours previously to the surgical procedures. Premedication was carried out using a mixture of diazepam (0.5 mg/kg, diazepam® Cristalia., Brazil) and tramadol (2 mg/kg, Tramal® Cristalia. Brazil) intramuscularly, followed by anesthetic induction using propofol (6 mg/kg, IV, Proporfol® Cristalia. Brazil). Anesthesia was maintained using constant rate infusion of propofol (0.5 mg/kg/min) associated to lidocaine chloride (Lidovet®, Bravet, Brazil) without epinephrine (1mg/kg/min).

After anesthetic induction, the animals were intubated using an 8-mm endotracheal tube with cuff. Aseptic surgical preparation was performed and local infiltrative anesthesia was carried out employing 0.4 mL of lidocaine chloride at each of the portal sites.

The animals were placed in dorsal recumbency with 45º head-down tilt (*Trendelenburg* position). A cutaneous stab incision was performed 15 cm cranially to the udder and 5 cm to the right of the midline for insertion of a 5-mm trocar using the blind technique without previous pneumoperitoneum creation. CO_2 pneumoperitoneum was then established using a 5-L/min flow rate and intra-abdominal pressure (IAP) was maintained at 8 mmHg. A 5-mm laparoscope was introduced into the abdominal cavity for prior inspection. A 10-mm portal was inserted in the opposite side and a third 5-mm trocar was introduced through the midline, 20 cm cranially to the udder, both using laparoscopic guidance.

The rigid endoscope was then transferred to the midline port. A 5-mm and a 10-mm atraumatic Babcock forceps were introduced through the first and second trocars, respectively, for safe manipulation of the uterus, oviducts and ovarian bursas. The ovaries were isolated and raised to the abdominal wall by grasping the mesovarium in order to avoid trauma to ovarian vessels and oviduct.

Prior to ovum pick-up, the ovaries were examined and the number of follicles measuring 2-8 mm in diameter was recorded. Following, an aspiration needle was introduced into the cavity in a position close to the ovary. Multiples punctures were performed by gently turning the ovaries around their axis. The needle was initially placed parallel to the ovarian surface allowing precise perforation of the follicles; in case such maneuver was not possible, the puncture was performed perpendicularly.

Vacuum pressure was adjusted to a maximum of 50 mmHg for follicular aspiration. A single lumen aspiration system composed of a 16 G needle with a short bevel, connected to a 50 cm length polytetrafluoroethylene cannula, was used. The cannula was then connected to a silicone cork, which was connected to the collection tube (50 mL). Vacuum was produced using an aspiration pump (Nevoni. Brazil), adapted to a sphygmomanometer. Previously to the oocytes aspiration, a washout was performed with the harvesting medium (PBS with heparin). Approximately 2 mL of the medium was left in order to receive the oocytes (Figure 1).

At the end of the aspirations, the ovaries were washed with 10 mL of 0.9% NaCl solution to remove clots, to minimize adhesion formation. Skin suture was carried out using interrupted Wolf pattern. No muscular layer or subcutaneous suture was performed. Finally, the surgical wound care was carried out using povidone-iodine. Additionally, a repellent ointment was used around the wound in order to prevent myiasis.

The surgical time was measured and after the procedure the sheep were placed in a clean and calm environment and were observed until normal standing and locomotion were noted.

Data were expressed as average ± standard deviation and were subjected to analysis of variance (Anova) and Tukey test ($P < 0.05$) using the GraphPad Prism 4™ statistics program.

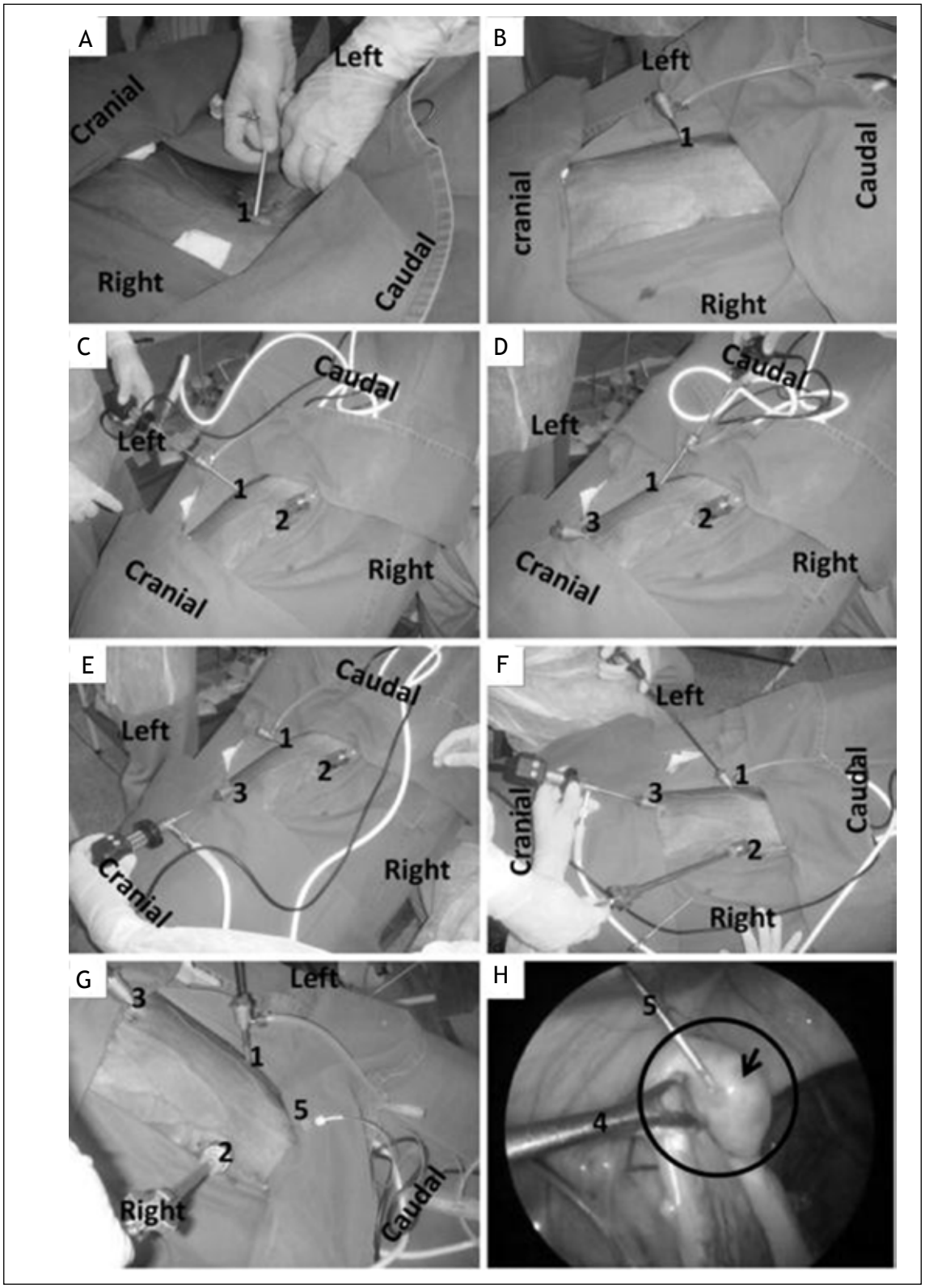


Figure 1 - Representation of the stages of follicular aspiration by laparoscopy (LOPU)
Legend: (A) placing the first trocar blindly with inflation valve (1). (B) Establishment of pneumoperitoneum by insufflating the first trocar (1). (C) Positioning of video-assisted second trocar (2). (D) video-assisted positioning of the third trocar (3). (E) Placement of the endoscope into the third trocar (3). (F) Positioning end of laparoscopic portals, and the camera in the third trocar (3) and atraumatic clamps the first and second trocar (1 and 2). (G) Placement of the aspiration needle cranial to the udder (5). (H) Inside view of the cavity with a seizure of the ovaries (circles) for an atraumatic clamps (4) and an ovarian follicle needle aspiration (5).
Source: Research data.

Results

Most ewes (72%) showed estrus 36 hours after progesterone removal. The average number of follicles verified and aspirated laparoscopically and the number of recovered oocytes were 13.32 ± 2.8, 10.35 ± 2.8 and 6.36 ± 2.0, respectively. A recovery rate of 61.39 ± 19.97% of aspirated follicles/recovered oocytes has observed. More than 85% of the recovered oocytes were considered good quality ones, which 47.6% of them were graded as I and 42.5% were graded as II in agreement with Hewitt and England (1997).

The 36-hour fasting and the 5-mmHg pneumoperitoneum were crucial for both avoiding reflux of ruminal content and providing adequate visualization and manipulation of the abdominal organs. Ruminal reflux was observed on 6 out of 60 laparoscopic sessions (10%). Adequate tracheal intubation allowed assisted breathing and avoided aspiration of ruminal content in case of reflux. The Trendelenburg positioning used in the current trial caused respiratory depression in 6.6% of the laparoscopic procedures. The learning curve was assessed by means of the full operative time (average of 26.75 ± 9.6 minutes). There was a significant decrease in surgical time ($P < 0.05$) from the first session on (Graph 1).

The major difficulties during the whole laparoscopic procedure were grasping the ovaries and puncturing the follicles. However, pneumoperitoneum and the amplified image transmitted by the video system improved the efficiency of the procedure.

The 16G needle with a short bevel, the single lumen aspiration system, the aspiration pump adapted to the sphygmomanometer and the vacuum pressure set to 50 mmHg were efficient for oocyte recovery. The bleeding observed following the follicular punctures was considered irrelevant. The removal of the ovarian clots using normal saline avoided postoperative adhesion formation in all animals, which was confirmed during the subsequent aspirations.

Regardless of the number of procedures performed, the ewes recovered uneventfully from the anesthesia and were able to remain standing and walk without difficulties. Moreover, no apparent painful discomfort or need for rescue analgesia was noted.

Discussion

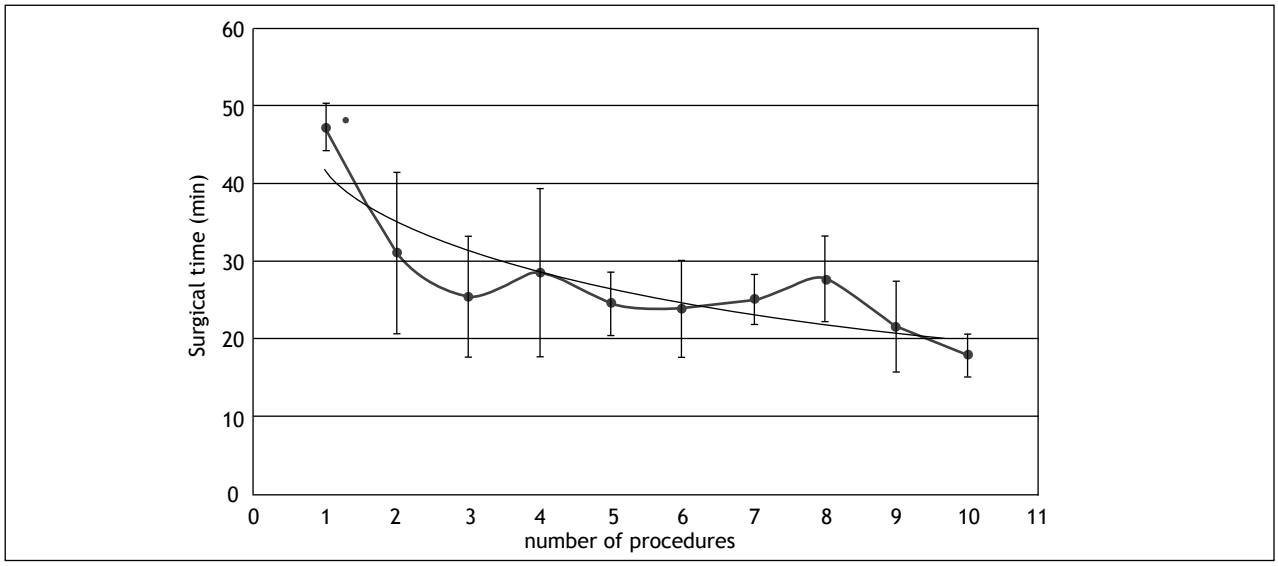
The results for ovarian stimulation and oocyte production obtained in the current trial were similar to those described in other studies that reported identification of 13.4 (BALDASSARE; KARATZAS, 2004) and 14.3 follicles/ewe (BASSO et al., 2008).

Ruminal reflux has been reported as a complication in sheep submitted to laparoscopy even after a 72-hour food fasting and 24-hour water fasting (BITTENCOURT et al., 2004). Such complication was attributed to the pneumoperitoneum and the anesthetic induction and maintenance using intravenous ketamine and acepromazine (BITTENCOURT et al., 2004). Regurgitation was not a major concern in the current study due to its low occurrence, which did not lead to any complication during and after the surgery.

Notwithstanding, the respiratory depression was easily managed using assisted ventilation and did not confer major risks to the patients. Reduced pulmonary volume, hypoxia, increased intrathoracic pressure, increased vascular resistance and increased cardiac stress have been reported as complications inherent to the head-down tilt positioning of the patient and pneumoperitoneum (DENNIS, 2006; CHANG; REGE, 2004; LÜTKE, 2001). Nevertheless, none of those complications were considered intolerable by the animals, probably due to the short operation time (DENNIS, 2006; CHANG; REGE, 2004; LÜTKE, 2001).

The mean operative time obtained in the current study was similar to those described in other trials involving ovum pick-up in goats and sheep: 35 minutes (CORDEIRO, 2006), 18 to 20 minutes (WIECZOREK et al., 2010). The mean surgical time was also comparable to the operative time obtained in liver biopsies in rams: 21 minutes (SILVA et al., 2006) and 23 minutes (DUARTE et al., 2009). In other study, between 120 and 150 minutes were spent to perform ovariectomies in bovines (BLEUL et al., 2005). Concerning such results, we hypothesize that the use of laparoscopic technique favored the execution of the procedures in a very convenient operative time. However, we highlight that the surgeon skills were important for obtaining such result.

No iatrogenic accidents happened in the current trial. The first trocar placement was performed blindly and without previous creation of



Graph 1 - Graphical demonstration of the learning curve of laparoscopic follicle aspiration in sheep, showing the time verses the number of surgical procedures

Note: Noting considerable decrease in surgical time from the first session (*p < 0.05).

Source: Research data.

pneumoperitoneum. Such maneuver was responsible for the increased operation time in the first sessions. However, blind introduction of the first trocar became technically easier and was carried out more quickly on the subsequent sessions. Iatrogenic accidents can occur during the surgery, especially laceration of internal organs during placement of the trocar (TABET et al.,2005). In some cases, creation of pneumoperitoneum prior to the first port placement, using the Veress needle technique, may be necessary in order to avoid organ perforation (DUARTE et al., 2009).

Adhesion formation was reported at the reproductive organs of small ruminants following surgical procedures (CORDEIRO, 2006; STANGL et al., 1999). Despite the fact that no classical anti-adhesiogenic fluids or barriers were employed in the current study, no postoperative adhesions were found. Heparinized solutions (BALDASSARE; KARATZAS, 2004; CORDEIRO, 2006) and carboxymethilcellulose (EWOLDT et al., 2004) were cited as classical anti-adhesion agents used routinely. The absence of adhesions obtained in the current study was attributed to the objective and meticulous manipulation of the reproductive tract and the mechanical removal of clots by spraying 0.9% NaCl solution on the ovarian surface.

The results of the current study reveal that the mean surgical times did not differ significantly by the second round of laparoscopic procedures. Furthermore, the development of skills regarding the studied procedure allowed collection of a satisfactory number of viable oocytes per sheep, even by a non-proficient surgeon.

Conclusions

The LOPU procedure showed a few complications, except by the respiratory depression caused by position, pneumoperitoneum, and ruminal reflux, which can bring negative consequences, specially when you do not have support and trained personnel. The short learning curve was short. The entry of the blind trocar, manipulating, apprehending and puncture of the ovaries, performed the most complex steps, but are overcome early in the training. More studies on surgical technique should be performed in order to avoid the complications that still exists in this procedure, in order to make feasible their use in the field.

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