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Aspiration of oocytes by laparoscopy for embryo transfer in goats: a review

Aspiración de ovocitos por laparoscopia para la transferencia de embriones en cabras:
una revisión

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ABSTRACT

The aspiration of oocytes by laparoscopy for the transfer of embryos is a technique used in the extraction of oocytes from a genetically superior animal, with the purpose of producing *in vitro* embryos to implant them in a female, which allows to increase the response to selection in a program of genetic improvement, by obtaining animals above the population average and because the generation interval can be reduced. Prior to the aspiration of the oocytes, it is necessary to prepare the donor and recipient females based on exogenous hormones, to achieve maximum production and extraction of oocytes in the same donor female and, consequently, a greater probable number of embryos produced and transferred. With the aim of describing the different protocols used in the aspiration of oocytes by the laparoscopic technique for the transfer of embryos in goats, the present literature review was carried out through scientific publications.

Keywords: Synchronization, superovulation, endoscopy, exogenous hormones, caprine.

RESUMEN

La aspiración de ovocitos por laparoscopia para la transferencia de embriones es una técnica utilizada en la extracción de ovocitos de un animal genéticamente superior, con la finalidad de producir embriones *in vitro* para implantarlos en una hembra, lo cual permite incrementar la respuesta a la selección en un programa de mejoramiento genético, al obtener animales superiores al promedio de la población y porque se puede reducir el intervalo de generación. Previo a la aspiración de los ovocitos, es necesario preparar a las hembras donantes y receptoras a base de hormonas exógenas, para lograr la producción y extracción máxima de ovocitos en una misma hembra donante y, por consecuencia, un mayor número probable de embriones producidos y transferidos. Con el objetivo de describir los diferentes protocolos utilizados en la aspiración de ovocitos por la técnica de laparoscopia para la transferencia de embriones en cabras, se realizó la presente revisión de literatura mediante publicaciones científicas.

Palabras clave: Sincronización, superovulación, endoscopia, hormonas exógenas, caprinos.

INTRODUCTION

Reproductive biotechnologies applied in small ruminants were first described in 1974. Laparoscopic oocyte aspiration (AOL, according its acronym in Spanish) is a reproductive biotechnology, which consists in aspirating the germ cells of a genetically superior animal (donor) to produce *in vitro* embryos and transfer them to another recipient animal (Lv *et al.*, 2010). This biotechnology is considered one of the most efficient methods for embryo transfer (ET), which has been shown to produce a greater number of offspring from females with outstanding genetic and productivity (Baldassarre, 2007).

The reproductive efficiency of goats depends on applied biotechnologies, as they are essential to sustain their global production (Souza-Fabjan *et al.*, 2014); that is why the AOL as biotechnology, has a key role in animal reproduction, through different breeding programs (Shin *et al.*, 2008). Although its application in assisted reproduction protocols in small ruminants has been limited, due to the high costs to perform this type of research (Freitas *et al.*, 2016). To obtain high quality oocytes in the TE in goats, the AOL has been used successfully (Leoni *et al.*, 2009), this is a minimally invasive technique, represents low risk for the development of adhesions and allows several oocyte collections of the same animal in a short period (Avelar *et al.*, 2012; Souza-Fabjan *et al.*, 2013). Therefore, the aim of this literature review was to describe the different protocols used in the technique of laparoscopic oocyte aspiration for the transfer of embryos in goats.

Synchronization of estrus and superovulation in goats

The synchronization of donor and recipient goats is important to perform the technique of laparoscopic oocyte aspiration. The protocols differ in the exogenous hormones used, but are similar in duration and application (Souza-Fabjan *et al.*, 2014).

Superovulation is achieved with exogenous hormones that promote follicular development and growth. To improve the collection of oocytes during the AOL in the ET, prior synchronization in the recipient and donor female is required; besides superovulating the donor female. Synchronization manipulates the estrous cycle and superovulation generates more oocytes for collection and evaluation. The synchronization of estrus (recipient-donor) is performed by intravaginal devices impregnated with P₄ (Abecia *et al.*, 2012).

The synchronization and superovulation protocols differ according to the suction means and the anesthetics to be used (Souza-Fabjan *et al.*, 2014, Table 1).

Table 1. Comparison of drugs, doses, materials and other values used for the technique of laparoscopic oocyte aspiration

Needle gauge	Anesthetic used	Aspiration medium	Vacuum pressure in the pump	Fluid speed	Author
20G	Ketamine (5 mg kg ⁻¹), diazepam (0.35 mg kg ⁻¹) and inhaled isoflurane.	TCM 199 supplemented with heparin (0.05 mg mL ⁻¹) and 1% fetal bovine serum.	--	50 a 70 drops min ⁻¹	Baldassarre, 2007
22G	Xylazine (0.5 mg 10 kg ⁻¹) and ketamine (25 mg 10 kg ⁻¹).	TCM 199 supplemented with HEPES (10 mM), heparin (20 IU mL ⁻¹) and gentamicin (40 mg mL ⁻¹).	30 mm Hg	7 a 7.5 mL min ⁻¹	Avelar <i>et al.</i> , 2012
22G	Thiopental (20 mg kg ⁻¹) and inhaled isoflurane at 3%.	TCM 199 supplemented with HEPES (10 mM), sodium pyruvate (0.022 mg mL ⁻¹), penicillin (10000 IU), streptomycin sulfate (10000 mg mL ⁻¹), amphotericin B (25 mg mL ⁻¹), FCS at 10% and heparin (20 IU mL ⁻¹).	30 mm Hg	7 a 7.5 mL min ⁻¹	Souza-Fabjan <i>et al.</i> , 2013
18G	Xylazine (0.5 mg 10 kg ⁻¹) and ketamine (25 mg 10 kg ⁻¹).	TCM 199 supplemented with HEPES (10mM), heparin (10 UI mL ⁻¹), gentamicin (4mg / mL) and BSA (1mg mL ⁻¹).	50 mm Hg	--	Souza-Fabjan <i>et al.</i> , 2014

G: Thickness of the needle. TCM: tissue Culture medium. HEPES: Buffer solution, N-2 hydroxyethylpiperazine-N'-2-ethane sulfonate. FCS: Fetal calf serum. BSA: Bovine serum albumin.

Baldassarre (2007) evaluated different protocols to synchronize donor females with intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (AMP, Veramix®, Upjohn Laboratories, Canada) for 10 days, and 48 hours before the AOL, applied 125 µg of cloprostenol (Estrumate®, Malinkrodt Laboratories, Canada). For super-ovulation, he compared two protocols; in the first, he injected follicle stimulating hormone (FSH, Folltropin®, Bioniche, Canada) and in the second, he combined FSH with equine chorionic gonadotropin (eCG, Pregnenol®, Bioniche, Canada), which he administered in a single dose 36 h before perform the AOL (protocol "OneShot", Baldassarre, 2007). The results were similar (P> 0.001) between the treatments of multiple injections and the "OneShot" treatments for the AOL (goats from 60 to 90 d and from 90 to 150 d of age: 84 and 80% recovery, respectively). However, for the young females, the protocols were identical to those used in the adults, due to the fact that they were not cycling and, therefore, estrous was not synchronized (intravaginal + luteolytic sponge, Baldassarre, 2007).

Avelar *et al.* (2012) synchronized donor goats using an intravaginal sponge with 60 mg of AMP (Syntex, Buenos Aires, Argentina) for 11 d, and on day eight, they injected 50 µg of d-cloprostenol (Ciosin, Coopers, São Paulo, Brazil). For ovarian stimulation, the goats were divided into three treatments: 1) 120 mg of NIH-FSH-P1 (standard swine FSH, National Institute of Health for standard FSH, Folltropin-V, Vetrepharm, Belleville, Canada), given in five injections (5D) intramuscularly (30, 30, 20, 20, and 20 mg, respectively) with intervals of 12 h; 2) 120 mg of NIH-FSH-P1 divided into three injections (3D) with intervals of 24 h (60, 40 and 20 mg, respectively); and 3) a single injection (1D) of 70 mg of NIH-FSH-P1 plus 200 IU of eCG (Novormon, Syntex, Buenos Aires, Argentina), 36 h before removing the sponge.

The results were similar ($P > 0.05$) between treatments for the number of follicles observed in each ovary; however, the highest percentage ($P < 0.05$) of oocyte recovery was obtained with the 5D treatment (84.1%, 211/251 females), with respect to that of the 3D treatments (68.2%, 182/267 females) and 1D (72.4%, 184/254 females), respectively (Avelar *et al.*, 2012).

The percentage of recovery of oocytes differs among authors, according to the number of females used per protocol and the response by animals or population (Table 2).

Table 2. Response variables obtained with the technique of oocyte aspiration by laparoscopy in goats.

Number of female	Race	Age of donor females	Follicles aspirated	Oocytes recovered	Percentage of recovery	Author
20		2 a 3 months	59.3 ± 28	49.7 ± 24*	84%	Baldassarre, 2007
36	--	3 a 5 months	34.4 ± 20	27.4 ± 14*	80%	
23		5 months	39.0 ± 4.5	28.4 ± 3.5*	73%	
21		>1 year	19.0 ± 1.4	15.9 ± 1.5*	84%	
16	Canindé	5 months	772	577**	74.7%	Avelar <i>et al.</i> , 2012
15	Canindé	2 a 4 years	245	182**	74.3 %	Souza-Fabjan <i>et al.</i> , 2013

Mean ± standard deviation. * Results per animal. ** Population results.

Souza-Fabjan *et al.* (2013) synchronized nulliparous and multiparous goats, using intravaginal sponges impregnated with 60 mg of AMP (Progespon, Syntex, Buenos Aires, Argentina) for 11 d. On day eight, they delivered 75 µg of d-cloprostenol (Prolise, Pfizer Animal Health, São Paulo, Brazil), and 36 h before removing the sponge, they applied a single dose of 70 mg of porcine FSH (FSHp, Folltropin-V Vetrepharm, Ontario, Canada) plus 200 IU equine chorionic gonadotropin (eCG, Novormon, Syntex). Oocyte collection was performed six days after the end of treatment. No differences were found ($P > 0.05$) for the response of estrus (40.0% vs. 84.7%), the onset of estrus (62.0 ± 15.5 vs. 50.7 ± 19.2 h, mean $0020 \pm EE$), the duration of estrus (25.0 ± 16.1 vs. 30.0 ± 15.1 h) and the percentage of ovulation (60.0% vs. 85.7%) in the study animals (Souza-Fabjan *et al.*, 2013).

Souza-Fabjan *et al.* (2014) synchronized goats with intravaginal sponges impregnated with 45 mg of fluoromethane acetate (AFG, Chronogest CR, Intervet Schering-Plow Animal Health, Angers, France) for 11 d; and on day eight, they injected a dose of prostaglandins (Cloprostenol 50 µg, Intervet Schering-Plow Animal Health); at the same time, they stimulated with 16 mg of FSHp (Stimufol, highly purified porcine FSH, kindly provided by Prof. JF Beckers, Liège, Belgium) at 12 h intervals with decreasing doses (4, 4, 3, 3, and 2 mg, respectively). With this protocol, the authors found similarities between the oocytes obtained by AOL and those originating from trace for intrinsic quality and development competence through parthenogenetic activation; however, they suggested that the oocytes obtained by AOL are less competent for fertilization under conditions of maturation and *in vitro* fertilization (Souza-Fabjan *et al.*, 2014).

Anakkul *et al.* (2013) synchronized goat recipients and donors, using intravaginal sponges with 65 mg of medroxyprogesterone acetate (AMP, Ovejero®, Spain) for 13 d, and an eCG injection (Folligon®, Intervet Schering-Plow Animal Health, Holanada). In estrus recipients, they administered a single dose of 200 IU of hCG (Chorulon®, Intervet Schering-Plow Animal Health, Holland) to induce ovulation. In addition to the P₄ analog (Sincro-gest sponges, 65 mg of Medroxyprogesterone, Ovejero®, Spain) in the donors, stimulated follicular development with seven doses of FSH (50, 25, 25, 25, 25, 25 and 25 mg, respectively Folltropin V®, Bioniche Animal Health, Vetrepharm, Canada) at 12-h intervals.

The first dose was applied on day 10 of the treatment. For the sixth and seventh doses, they injected 125 µg mL⁻¹ of cloprostenol (Estrumate®, Schering-Plow Animal Health, NJ, USA). They detected estrus every 6 h and to induce ovulation, they applied 200 IU of hCG (Chorulon®, Intervet Schering-Plow Animal Health, Holland). Thirty embryos from 11 donors (Black Bengal: BB=7, 50%; Australian Melaan: AA=4.0%, recovery percentages in three donors) were transferred in 30 recipient females. 30% of gestation was obtained (9/30) from where nine offspring were born and one of them presented black color.

The results suggest that artificial insemination and TE by laparoscopy can be combined to improve the genetic potential that codes for the black color of the skin (Anakkul *et al.*, 2013).

Perera *et al.* (2008) synchronized donor goats with vaginal devices (45 mg, Cronolone, Intervet) for 17 d; applied 2.5 ml of FSHp (Folltropin-V, 20 mg/ml NIH-FSH-P1, BIONICHE, Canada); and ovine FSH (FSHo; Ovagen™, 0.88 mg/mL NIADDK-oFSH-17-Standard, ICPbio Limited, New Zealanda) on eighth day. In addition, they injected 300 IU of eCG (Folligon, Intervet International BV, Boxmeer-Holland), during the night of the eight. They stimulated folliculogenesis and follicular maturation with injections of 1.25 ml of FSHp or FSHo in the morning and on the evening of day 9 and 10. On the morning of ninth day, they injected 197 mg of prostaglandin F_{2α} (PGF_{2α}, cloprostenol sodium; PGF Veyx fort, Veyx Pharma, Schwarzenborn) and removed the vaginal devices on the night of day 10. On the morning of day 11, they applied 1.25 ml of FSHp and FSHo, and at night they applied 1 ml of luteinizing hormone-releasing hormone (LHRH; µL/ml, Depherelin Veyx Pharma, Schwarzenborn). After this injection, they kept the goats in contact with a goat for natural hunts for 48 h (Figure 1).

No significant differences were found in the number of embryos per animal in both protocols (FSHp: 4.3 ± 2.0 and FSHo: 4.25 ± 2.0, respectively). Possibly the protocols are adequate; nevertheless, it is necessary to perfect the AOL technique (Perera *et al.*, 2008).

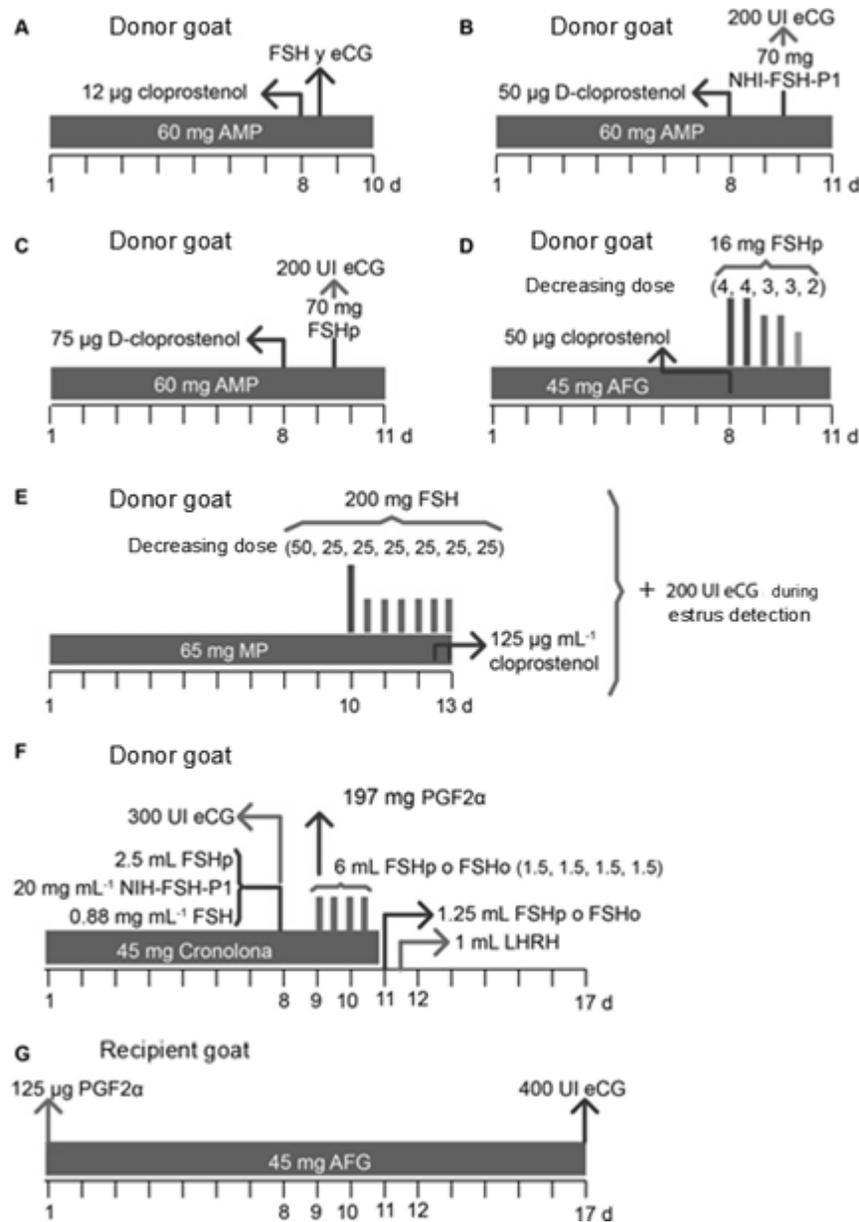


Figure 1. Synchronization and superovulation protocols in goats

(A) Baldassarre, 2007; (B) Avelar *et al.*, 2012; (C) Souza-Fabjan *et al.*, 2013; (D) Souza-Fabjan *et al.*, 2014; (E) Anakkul *et al.*, 2013; (F) and (G) Perera *et al.*, 2008. AMP: medroxyprogesterone acetate, AFG: fluoromethane acetate, MP: mexodroxyprogesterone, FSH: Follicle stimulating hormone, NHI-FSH-P1: Standard porcine follicle stimulating hormone, FSHp: Porcine follicle stimulating hormone, FSHo: Ovine follicle stimulating hormone, eCG: Equine chorionic gonadotropin hormone, hCG: Human chorionic gonadotropin hormone, PGF_{2α}: Prostaglandin F2 alpha.

Laparoscopic oocyte aspiration (AOL)

Laparoscopy allows vision and manipulation of the abdomino-pelvic cavity by means of the laparoscope, which is introduced through a small incision and consists of a light source transmitted by an optical fiber; by means of which a monitor is projected (Baldassarre, 2007). To perform the AOL technique, donor females are required to be

fasted for at least 24 hours beforehand to reduce the risk of accidental punctures in the intestines, rumen or urinary bladder during the procedure. The female is depilated and disinfected with iodized alcohol in the abdominal area, placed on a dorsal decubitus stretcher to tilt it to an angle of 30 to 45 °, so that the viscera move in the cranial direction and allow to visualize the reproductive tract of the female (Alexander *et al.*, 2010). The AOL requires highly qualified personnel, a laparoscopic team consisting of a laparoscope of 5 mm in diameter, an atraumatic clamp and an aspiration pipette of 3.5 mm in diameter, with their respective trocars (Baldassare, 2007).

The endoscope is inserted 2 to 3 cm cranial to the mammary gland, and 2 to 3 cm to the left of the midline in the abdominal cavity through a first trocar; a second trocar is inserted in the right side of the abdomen (opposite to the first) to introduce the applicator-tracer, with which the uterus is located. Next, a third trocar is inserted in the midline, in order to pass the oocyte retrieval needle (Souza-Fabjan *et al.*, 2014). Once the three trocars were inserted and the laparoscope was inserted, the atraumatic clamp and the aspiration pipette, follicular puncture was performed (Cortez-Romero *et al.*, 2011, Figure 2).

To perform it, the ovary is discovered by lifting the fimbria with the atraumatic clamp and pulling in different directions, to expose the different areas on its surface. The procedure is repeated in both ovaries and before removing the equipment, both ovaries are washed with heparinized physiological solution at 37 °C (Baldassare, 2007).

The embryo transfer method requires synchronization between the donor and the recipient, ensuring that the embryo is collected in the appropriate stage of development (morula or early blastocyst), and then transferred to the recipient in the proper state of the estrous cycle. Laparoscopic methods of embryo collection and transfer have replaced surgical laparotomy methods, reducing the risk of postoperative complications of the reproductive tract (Garza-Arredondo *et al.*, 2015).

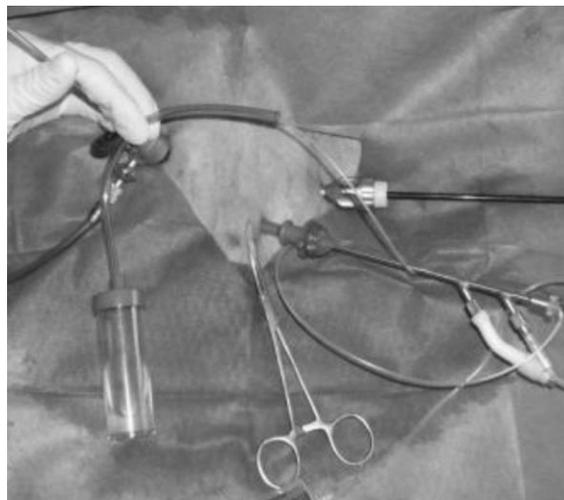


Figure 2. Aspiration of oocytes by laparoscopy (Adapted from Cortez-Romero *et al.*, 2011).

The technique of laparoscopy is convenient for ovaries with a small number of follicles, and has the advantage of repeating aspirations at short intervals. For ovaries that present a large number of follicles after stimulation by gonadotropins, abdominal laparotomy under general anesthesia makes aspiration more efficient, and the recovery rate of oocytes is higher (Córdova-Izquierdo *et al.*, 2008).

CONCLUSIONS

The amount of follicles aspirated and oocytes recovered varies due to the number of animals used, the age, breed and prolificacy of the females.

The amount and percentage of recovered oocytes differs according to the age of the donor female and the velocity of the fluid used for the aspiration of the oocytes; since, at a younger age, more oocytes are obtained, and at a lower aspiration rate, a higher percentage of oocytes with better quality is obtained for the transfer of embryos in goats.

The use of AOL has an additional advantage over traditional protocols, because its application in the *in vitro* production of embryos, by means of oocytes collected by laparoscopy, is very important to promote assisted reproduction in goats. On the contrary, a disadvantage is that the technique involves training, infrastructure and highly specialized and expensive equipment.

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