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Evolution of follicular aspiration for *in vitro* embryo production in ruminants

Evolución de la aspiración folicular para producción *in vitro* de embriones en rumiantes

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ABSTRACT

Since the birth of the first offspring generated from *in vitro* embryo production (IVP) in 1982, advances in this technology have not stopped. IVPhas been so successful that it has been adapted to various species, both productive and wild. Undoubtedly, the greatest success of IVP has been in cattle, according to data reported by the International Embryo Technology Society (IETS). This success is largely due to the evolution of *in vitro* culture systems and the efficient and minimally invasive collection of oocytes from living donors. In the last 3 decades, advances in ovarian physiology, development of ultrasonography, types of needles, aspiration pressure, used genotypes, have allowed follicular aspiration to be a technique that can be developed at the field level, with results better each time without affecting the well-being of the donors. In this review, the evolution of the follicular aspiration technique in ruminants will be described with emphasis on its variants and new strategies to improve oocyte quality.
Keywords: *In vitro* produced embryos, ovarian follicular aspiration, ruminants.

RESUMEN

Desde que, en 1982 se consiguió el nacimiento de la primera cría generada a partir de la producción *in vitro* de embriones (PIV), los avances en esta tecnología no se han detenido. La PIV ha sido tan exitosa que se ha adaptado a diversas especies, tanto productivas como de fauna silvestre. Sin lugar a duda el mayor éxito de la PIV ha sido en el ganado bovino, según los datos reportados por la Sociedad Internacional de Tecnologías Embrionarias (IETS) durante el año 2020. Este éxito se debe en gran parte a la evolución de los sistemas de cultivo *in vitro* y a la colección eficiente y poco invasiva de ovocitos a partir de donantes vivas. En las últimas 3 décadas el avance en la fisiología ovárica, en el desarrollo de la ultrasonografía, tipos de agujas, presión de aspiración y genotipos utilizados, ha permitido que la aspiración folicular ovárica sea una técnica que se puede desarrollar a nivel de campo, con resultados cada vez mejores y sin afectar

el bienestar de las donantes. En esta revisión se describirá la evolución de la técnica de aspiración folicular en rumiantes con énfasis en sus variantes y las nuevas estrategias para mejorar la calidad de los ovocitos.
Palabras clave: embriones producidos *in vitro*, aspiración folicular ovárica, rumiantes.

INTRODUCTION

Currently, embryo production (IVP) and embryo transfer (ET) have had a great impact on animal production. In the case of cattle, IVP is widely applied and the vast majority of embryos produced worldwide are generated by this technology according to data reported by the International Embryo Technology Society (IETS) (Viana, 2021) (Table 1). One of the reasons for the wide application of IVP in cattle, with respect to other species, is the advance in other reproductive technologies such as follicular aspiration (of cumulus-oocyte complexes from 2-8 mm follicles) which has allowed the repeated and efficient collection of immature oocytes from live donors (Galli *et al*., 2001) without affecting the animal welfare of the donors (Chastant-Maillard *et al*., 2003; Petyim *et al*., 2007; Currin *et al*., 2017). It is important to highlight that there are reports of programs with two ovum pick up (OPU) sessions per donor per week (Petyim *et al*., 2003), which allows maximizing the genetic potential of the donors (Pontes *et al*., 2011).

Table 1. *In vivo* and *in vitro* production of transferable bovine embryos during 2020, by region (Viana, 2021)

Region	Africa	Asia	Europe	North America	Oceania	South America	**Total**
IVD	2.763	0	126.491	196.704	4.211	31.559	**361.728**
IVP	4.977	0	47.470	578.995	14.345	500.397	**1 156.422**

IVD: *in vivo* produced embryos
IVP: *in vitro* produced embryos

IVP requires several techniques such as donor oocyte collection, *in vitro* maturation, in vitro fertilization and *in vitro* culture until embryo production at the blastocyst stage (Galli *et al*., 2001; Tamassia *et al*., 2003). Oocyte collection can be performed by ultrasound-guided follicular aspiration (OPU) in larger species such as cattle or by laparoscopic follicular aspiration (LOPU) in small ruminants (sheep, goats, deer and calves) (Baldassarre, 2021). The main objective of both technologies is to collect oocytes (mainly immature) from genetically superior donors to be fertilized *in vitro* with elite sires and generate embryos, which can be transferred to previously synchronized recipients (Merton *et al*., 2003). However, they can also be used for conservation and/or genetic rescue purposes (Ruiz *et al*., 2013; Baldassarre, 2021). These follicular aspiration techniques are not very invasive and the repeated recovery of donor oocytes allows multiple crosses, as well as reducing the generation interval in genetic improvement programs, producing more embryos and pregnancies per donor than by the multi-

ovulation technique and *in vivo* embryo production (IVD) (Merton *et al*., 2003; Baldassarre *et al*., 2007).

GENERALITIES OF THE OPU TECHNIQUE

The OPU system is composed of three parts: an ultrasound with a linear (rectal) or micro convex (5-7.5 MHz) transducer associated with a device for its fixation (aspiration gun), an aspiration pump and a needle connected to the aspiration system (Bols *et al*., 1996). For follicular aspiration, transducers that increase the resolution and size of the figures are required for better manipulation of the ovaries (Ginther, 2014). The microconvex transducer is the most commonly used, it has an ultrasonographic field of 150°, it can be of 5-7.5 MHz, preferring the 7.5 MHz, since it has better resolution and allows easier localization of small follicles. The 150° ultrasonographic field helps to facilitate manipulation of the ovaries and to locate follicles in the puncture line, allowing the use of shorter needles that enter directly into the ultrasound field, without losing usable portions of the needle (Ginther, 2014). The needle is attached and directed into the ultrasound field through a guide that is made up of a thin stainless steel tube. Inside the steel tube is the suction system hose, which is attached to the needle and the steel tube through a silicone tube that, in conjunction with a connecting piece, creates a rigid structure that allows for forward and backward movement of the needle, in addition to being able to easily replace the blunt needle with a new one (Palma, 2001).

The transducer and needle guide are inserted into the aspiration gun, which has a handle that gives the operator the opportunity to fix the OPU device inside the vagina by gently pressing the handle with the right hand, leaving the left hand free to manipulate the ovary (Palma, 2001). Cows used for OPU should be immobilized and/or may be sedated if necessary. Feces are removed from the rectum during palpation and epidural anesthesia with 2 % lidocaine is performed to avoid discomfort to the animal and rectum movements during the procedure. The vulva and perineum are cleaned and disinfected before inserting the OPU device. The device has a handle by which it can be manipulated with one hand outside the cow. The transducer head is fixed in a dorsal cranial position at the bottom of the vagina, above the cervix (Palma, 2001).

Technical Aspects

After the initial development of the technique, several studies have been carried out to improve follicular aspiration techniques, which are currently widely used as a reproductive tool for genetic breeding programs. Among the main technical aspects that have been worked on are needle type, bevel type, vacuum pressure and transducer type (Bols *et al.,* 1996; Bols *et al.,*1997; Palma, 2001; Van Wagtendonk-de Leeuw, 2006).

Regarding the needle type, the needle gauge has been evaluated using 18G, 19G and 21G needles, and it has been observed that the diameter with which the greatest recovery is obtained is 18G. However, this is relative, since the suction pressure has a greater influence on the quantity and integrity of the oocytes recovered (Bols *et al*., 1996). Initially, long needles were used, 50 to 65 centimeters, with an external diameter of 1-1.5 mm and a short bevel (Bols *et al*., 1997) (Figure 1). The major disadvantage of long needles is that they dull quickly and require sharpening, but do not reach the initial state of sharpness, which is important since the sharpness of the needle is essential for the technique to be successful (Bols *et al.,* 1996). These long needles are expensive and present a large dead space in their lumen, leaving the oocytes in an unfavorable environment for a long period, and large amounts of medium are needed to clean them (Palma, 2001).

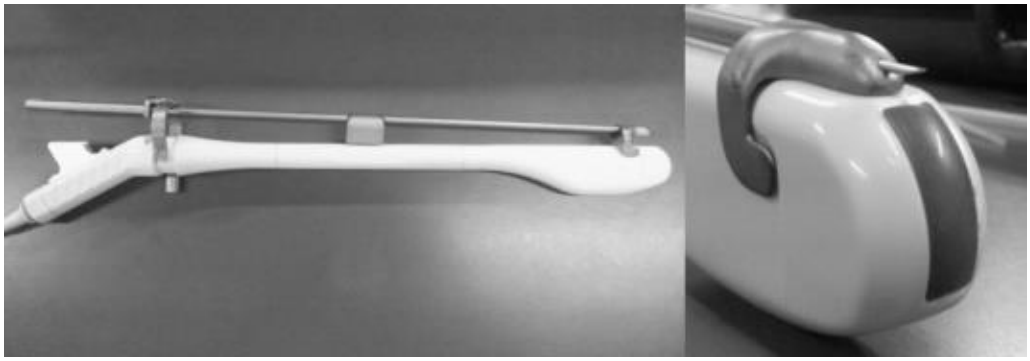

Figure 1. Device for OPU with long needle

The simplification of the devices used for OPU to the use of disposable needles opened a new landscape for the technique, since a blunt needle can be easily changed because they are inexpensive. Different diameters and lengths of sterile disposable needles are available on the market. Dead space is minimal, so oocytes can be retrieved under more favorable conditions (Bols *et al*., 1997). The risk of contamination between one donor and another is reduced, and the time that oocyte *cumulus* complexes (OCCs) are in an unfavorable environment is minimized (Van Wagtendonk-de Leeuw, 2006).

Another aspect studied was the influence of bevel type, short or long (Figure 2), on oocyte recovery. Although similar recovery was observed for both bevel types, suction pressure was found to significantly affect oocyte recovery and integrity when using short bevel needles (Bols *et al*., 1997). Commercial needles are now available for OPU, which have an extension that connects directly to the aspiration line (Figure 3).

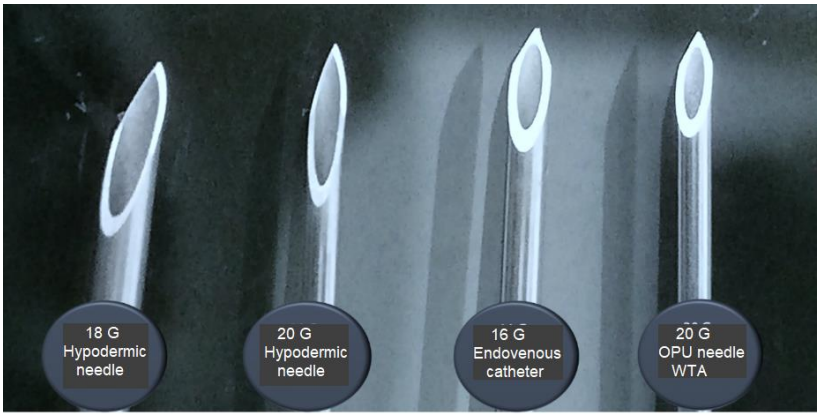

Figure 2. Short and long bevels according to needle type

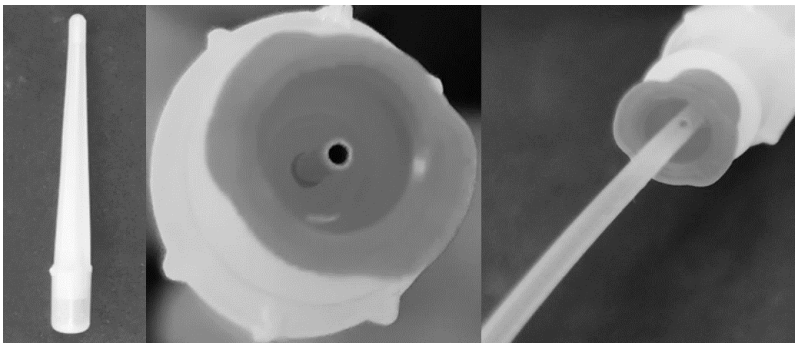

Figure 3. WTA™ needle with extension to connect the suction line

Regarding vacuum pressure, it has been observed that it varies according to the type of needle and bevel used; however, it was found that the higher the pressure, the greater the oocyte recovery, but also the number of naked oocytes increases (Palma, 2001). For disposable needles, the recommended suction pressure ranges from 70 to 130 mm Hg, since reducing the vacuum pressure increases the integrity of the OCCs and this increases blastocyst production (Bols *et al*., 1996; Bols *et al*., 1997).

Since 1984, when the first reports of reproductive evaluation in cattle were generated (Reeves *et al*., 1984; Pierson & Ginther*.,* 1984), transrectal ultrasonography has become a basic tool in bovine reproduction (Ginther *et al*., 2014), due to this, at first it was tried to use a rectal linear transducer, this because the vast majority of professionals in the area of reproduction in large species, have an ultrasound with rectal linear transducer (Van Wagtendonk-de Leeuw, 2006) (Figure 4A). When comparing the use of a linear transducer with a microconvex transducer (Figure 4B), it was observed that there was no difference in detecting large follicles (<5 mm), however, when follicles were small (>5 mm) there was a 12 % decrease in visualization with the linear transducer. As for oocyte recovery, with the microconvex transducer there was a doubling of oocyte recovery with respect to the linear transducer, although this difference was not statistically significant (Bols *et al*.,

2004). This difference is due to the fact that the area to manipulate the ovary to perform the puncture is larger with the microconvex transducer compared to the linear transducer (Figure 4).

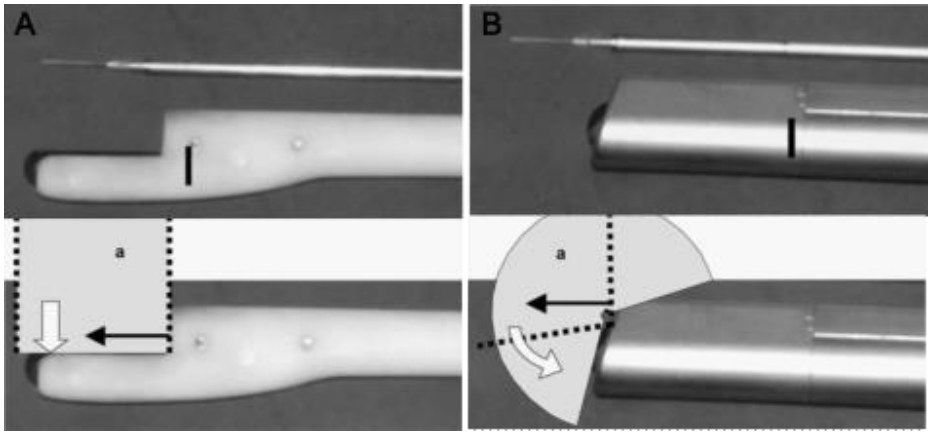

Figure 4. OPU devices. A) Device and puncture area with rectal linear transducer. B) Device and puncture area with microconvex transducer (Bols *et al*., 2004)

Currently most devices use a microconvex transducer (Xavier *et al*., 2023; Camargo *et al*., 2019; de Carvalho *et al*., 2019), there is even a form-fitted microconvex transducer for the OPU device (Figure 5).

Figure 5. Shaped microconvex transducer for OPU device

Biological aspects

Among the biological aspects related to the OPU technique are the animal genotype, ovarian stimulation, timing and frequency of OPU, individual donor response, physiological state of the donor (empty/gestation; lactating/non-lactating), donor age (cow, heifer, calf), time of year and the technician experience to perform OPU (Palma, 2001; Tamassia *et al*., 2003; Camargo *et al*., 2005; Van Wagtendonk-de Leeuw, 2006; Takuma *et al*., 2010; Vieira *et al*., 2014). Hormonal stimulation is undoubtedly one of the most important improvements with respect to oocyte quality and blastocyst production *in vitro*.

Additionally, the use of hormones for follicular stimulation is an advantage that OPU-IVP has over IVD. Follicle stimulating hormone (FSH) and equine chorionic gonadotropin (eCG) can be used for follicular stimulation (Aller *et al*., 2012) and although this practice is mainly done in *Bos taurus* cattle (Presicce *et al*., 2011; Aller *et al.,* 2012; Chasombat *et al*., 2013; Vieira *et al.,* 2014), good results have also been seen when applied in *Bos indicus* cattle (Table 2). However, large-scale IVP programs have also been observed with *Bos indicus* (Nelore) cattle in which donors were not stimulated and had efficient embryo production (Pontes *et al*., 2011).

On the other hand, in order to make intensive embryo production programs, IVP has been combined with IVD, where good results (5.1 embryos per wash and 3.2 freezable embryos) have been obtained by starting superovulation 2 days after OPU using Nelore donors (Surjus *et al*., 2014). On the other hand, donor genotype has a lot to do with follicular population. *Bos indicus* donors are known to exceed 2 to 4 times the amounts of oocytes collected by OPU compared to *Bos taurus* donors (Baruselli *et al*., 2015). It has also been observed that *Bos indicus* donors have more follicular surges and a larger population of antral follicles larger than 5 mm in diameter compared to *Bos taurus* (Silva-Santos *et al*., 2011).

Table 2. Summary of progress in the follicular aspiration technique in cattle

Use of hormones to stimulate	Needle L = long C= short	Pressure mmHg	Transducer	No. Oocytes[a]	Recovery (%)[b]	Viable oocytes(%)[c]	Viable embryos *[d]	Reference
Bos Taurus								
No	23G L	-	Vaginal	13.0	50.4	-	2.2	Pieterse *et al.*, 1991
No	20G L	40-50	Vaginal	8.0	55.1	77.61	1.0	Kruip *et al.*, 1994
FSH 24-30 mg	17G L	75-100	Convex	8.6	69.9	-	1.3	Looney *et al.*, 1994
FSH 75 UI	17G L	70	Vaginal	9.5	-	80.0	2.7	Rocha *et al.*, 1998
No	20G C	50	Vaginal	3.9	58.5	86.0	-	Petyim *et al.*, 2003
No	19G C	-	Linear	0.9	9.2	73.0	-	Bols *et al.*, 2004
No	19G C	-	Convex	11.4	-	70.1	2.1	Pontes *et al.*, 2010
eCG 1600 UI	20G C	65	Vaginal	2.2	34.2	73.8	-	Aller *et al.*, 2012
FSH 200 mg	20G C	85-90	Convex	9.9	61.1	83.9	4.4	Vieira *et al.* 2014
No	20G C	68	Convex	17.3	81.8	71.1	3.0	Guerreiro *et al.*, 2014
No	20G C	60-70	Convex	14.6	60.4	74.1	0.7	Sales *et al.*, 2015
No	20G C	68	Convex	9.2	36.9	51.08	0.5	Batista *et al.* 2016
No	18G C	90	Convex	6.3	-	71.2	5.2	Oliveira et al., 2019
FSH 70 UI	18G C	70	-	26.4	64.6	96.5	7.1	Simmons *et al.*, 2023
Bos indicus								
FSH 75 UI	17G L	70	Vaginal	10.3	-	83.3	5.4	Rocha *et al.*, 1998
No	20G C	80	Vaginal	8.9	61.3	74.1	1.1	Viana *et al.*, 2004
No	20G C	80	Vaginal	7.0	69.3	62.1	-	Viana *et al.*, 2010
No	19G C	-	Convex	17.1	-	70.1	3.2	Pontes *et al.*, 2010
FSH 100 mg	17G L	120	Vaginal	20.8	91.6	64.4	6.8	Chasombat *et al.*, 2013
No	20G C	68	Convex	45.3	77.5	50.7	7.0	Guerreiro *et al.*, 2014
No	20G C	60-70	Convex	22.8	88.8	84.9	3.8	Sales *et al.*, 2015
No	20G C	68	Convex	29.9	63.6	60.5	9.3	Batista *et al.*, 2016
No	18G C	90	Convex	10.0	-	78.8	10.2	Oliveira *et al.*, 2019
No	18G C	60-80	Convex	30.1	-	85.7	10.3	García *et al.*, 2020
No	20G C	90-100	Convex	58.6	78.3	74.2	9.7	de Silva *et al.* 2022
No	20G C	80	Convex	24.7	88.4	78.7	9.6	Xavier *et al.*, 2023

* No. of oocytes retrieved over No. of follicle aspirated
** No. of viable oocytes out of No. of oocytes retrieved
*** No. of viable embryos per aspiration

Regarding strategies to increase IVP, it has been shown that oocytes matured *in vivo* are more competent compared to those matured *in vitro* (Van de Leemput *et al*., 1999; Camargo *et al*., 2019). In 1994 it was reported that bovine oocytes aspirated from dominant follicles before the peak of luteinizing hormone (LH) show alterations in their nuclear and cytoplasmic morphology that, according to the authors, are a prerequisite for the acquisition of their full competence.

This would indicate that not only the final maturation of the oocyte is transcendental, but that the process occurs between the LH peak and ovulation. Furthermore, the period preceding the LH peak may be important in the establishment of developmental competence (Assey *et al*., 1994).

In a 2014 work, 54.9 % blastocysts were successfully obtained with sexed semen in Holstein cattle from *in vivo* matured oocytes (Figure 6) and synchronization of the follicular surge by dominant follicle ablation (Matoba *et al*., 2014). In 2019, when working IVP in Black Japanese cattle, it was concluded that higher quality embryos can be generated efficiently when using *in vivo* matured oocytes collected by OPU compared to immature oocytes (Egashira *et al.,* 2019).

Among the strategies for selecting oocyte donors for IVP is the evaluation of plasma anti-Müllerian hormone (AMH) levels. Donors classified as having high AMH levels produced greater numbers of embryos per OPU compared to those classified as having low AMH levels, regardless of whether they were *Bos taurus* or *Bos indicus*. With these results, it was concluded that plasma AMH levels are an accurate endocrine marker for the selection of oocyte donors (Guerreiro *et al*., 2014).

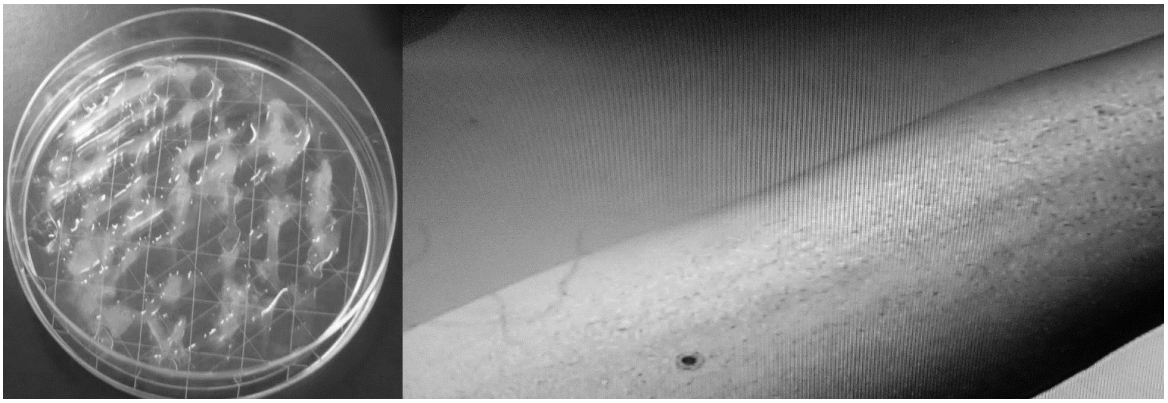

Figure 6. Search for matured oocytes *in vivo*

Currently, color Doppler ultrasonography has had multiple applications in the reproductive evaluation of cattle. Ovarian irrigation is known to be very important for oocyte protection and formation (blood-follicular barrier), follicular fluid formation and nutrient transport (Da Broi *et al*., 2018). Recently it has been found that ovarian irrigation plays a very important role in IVP, it has been observed that ovaries of oocyte donors with an irrigation between 50 and 70 % approximately, produce a greater number of embryos than donors with ovarian irrigation of about 30 % (Figure 7). In this work it was observed that donors with ovarian irrigation of ~70 % had a blastocyst production of 38 %, while donors with irrigation of ~50 % produced 30 % blastocysts and those with irrigation of ~30 % produced 18 % blastocysts (Villaseñor-González *et al*., 2021).

FOLLICULAR ASPIRATION IN SMALL RUMINANTS

Sheep embryo production

Currently, IVD is the main method used to generate embryos in small ruminants. Embryo collection is performed by the laparotomy surgical method, however, this technique has several drawbacks, among which adhesions, postoperative trauma, and the prolonged time between one procedure and another stand out (Jorge-Neto *et al*., 2018), which means that the process can only be repeated 4 to 5 times in the vast majority of donors.

As an alternative to surgical embryo collection, in recent years many researchers have worked on the development of a non-surgical technique (dilating the cervix), using substances such as misoprostol, prostaglandin F2α and estrogens mainly. Although there are promising results (complete penetration of the cervix ranging from 27-78 % of donors, lavage medium recovery of about 88 to 91 %) there is still a lack of further development of the technique so that it can replace surgical lavage (Fonseca *et al*., 2019; da Fonseca *et al*., 2019).

Considering the above, IVP in small ruminants also constitutes a tool for genetic improvement and for the conservation of gametes and embryos, through LOPU. This tool is highly repeatable, minimally invasive (since it does not generate adhesions) and with a short execution time (approximately 15 minutes per donor). The period between procedures can be 14 days, which is considerably less compared to 60 days to obtain embryos by IVD (Jorge-Neto *et al*., 2018). However, highly trained personnel and an in vitro embryo production laboratory are required.

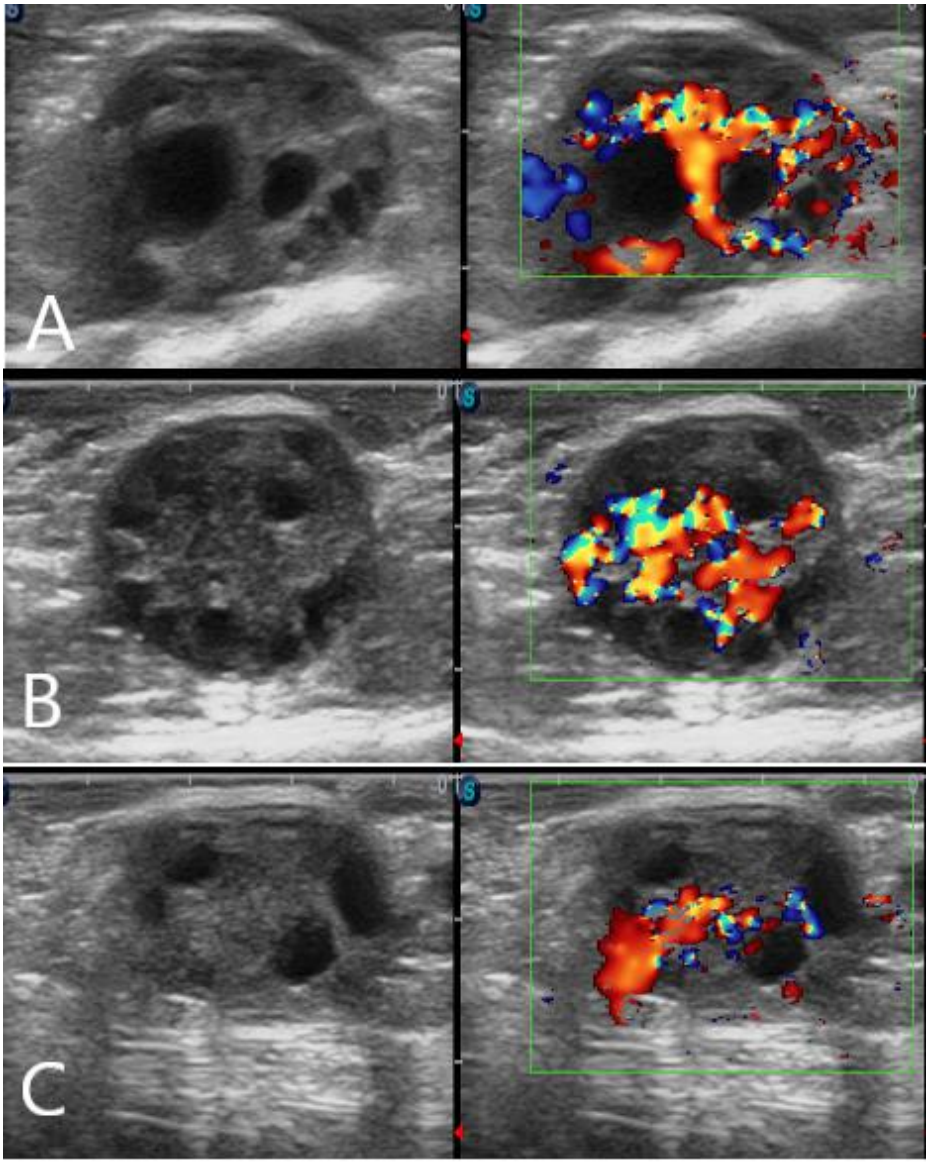

Figure 7. Evaluation of the degree of ovarian irrigation by Color Doppler ultrasonography. A) Ovary with ~70 % irrigation; B) Ovary with ~50 % irrigation; C) Ovary with ~30 % irrigation (Villaseñor-González *et al*., 2021)

According to the statistics shown by IETS (Table 3), IVP is used commercially in sheep, but to a lesser degree compared to IVD, which occurs mainly in North America. Although in the case of goats, an upward trend in the use of IVP can be observed, as reported by IETS in 2020 (Viana, 2021).

Table 3. *In vivo* and *in vitro* sheep embryo production in 2020 by region (Viana, 2021)

Region	Sheep		Goats	
	IVD	IVP	IVD	IVP
Africa	0	0	0	0
Asia	0	0	0	0
Europe	966	0	346	0
North America	9.204	141	10.757	2.275
Oceania	12.427	0	1.890	0
South America	7.222	0	184	0
Total 2020	**29.819**	**141**	**13.177**	**2.275**
Total 2019	**22.374**	**1.137**	**8.725**	**748**

IVD: *in vivo* produced embryos
IVP: embryos produced *in vitro*

Overview of LOPU

LOPU is a very reliable and efficient technique for collecting high-quality oocytes from live animals, and in some cases older animals, which allows its use for IVP. This technique has application in sheep, goats, cattle, buffalo (Jorge-Neto *et al*., 2018; Baldassarre, 2021) and cervids (Locatelli *et al*., 2006; Baldasssarre, 2021). Repeat LOPU in the same donor does not cause sequelae with impact on the reproductive life of the female, even when performed in prepubertal or wild animals. Another advantage of LOPU is that it can be performed in small ruminants even when they are out of reproductive season, in addition to being able to be applied in prepubertal animals, including cattle and buffaloes (Jorge-Neto *et al*., 2018; Currin *et al*., 2021; Sousa *et al*., 2022).

In small ruminants, LOPU associated with IVP and ET is the most efficient way to produce a higher number of offspring from females of outstanding genetics and productivity. In breeding programs, this allows increasing the selection differential as well as the generation interval and thus accelerate the speed of genetic progress.

LOPU is performed under anesthesia with ketamine (5 mg/kg IM) plus xylazine (0.2 mg/kg IM) after a 24-hour fast. Once anesthetized, the donor is placed on the laparoscopic table, shaved and disinfected in the abdominal area immediately cranial to the udder. The donor is placed head down at an angle of approximately 45-60 degrees, so that the digestive organs rest on the diaphragm and allow the uterus and ovaries to be seen. This is done using a 0° endoscope (preferably 5 mm in diameter), associated with three trocars (one trocar for the endoscope, one for an atraumatic forceps and one for the aspiration mandrel, all preferably 5 mm) (Figure 8) to gain access to the abdominal cavity, which allows us to minimize the degree of surgical trauma.

Pneumoperitoneum is performed to increase visualization by insufflating the abdominal cavity with CO_2. The suction mandrel is composed of a 5 mm diameter stainless steel or acrylic tube, attached to a 20G needle which is connected to a siliconized hose that flows into a 50 mL collection tube which in turn is connected to the vacuum pump (Figure 9).

The vacuum pressure is set at a rate of 50-70 drops per minute. Once the 3 trocars have been properly inserted and the laparoscope, the atraumatic grasping forceps and the aspiration pipette hmave been introduced through them, the follicular puncture is performed. To perform the follicular puncture, the ovary is grasped with the atraumatic forceps and rotated in different directions to see the entire surface of the ovary and thus puncture all the follicles larger than 2 mm in diameter. The procedure is repeated in both ovaries, and the ovaries are washed with heparinized physiological solution to eliminate any remaining blood and to prevent adhesions from forming (Baldassarre, 2021).

In addition, hormonal stimulation with FSH is required in donors because the ovaries are generally small and in order to facilitate puncture (Palma *et al*., 2001; Baldassarre *et al*., 2007; Baldasarre, 2021). It has been reported that stimulation with FSH every 8 hours starting 36 hours before LOPU and changing the last application to eCG in calves produced embryos similar to those obtained with adult donors (Baldassarre *et al*., 2018). It is important to apply prostaglandin F2α prior to LOPU because, if any corpus luteum is present, bleeding is greater due to the high irrigation in this structure (Vilá *et al*., 2007).

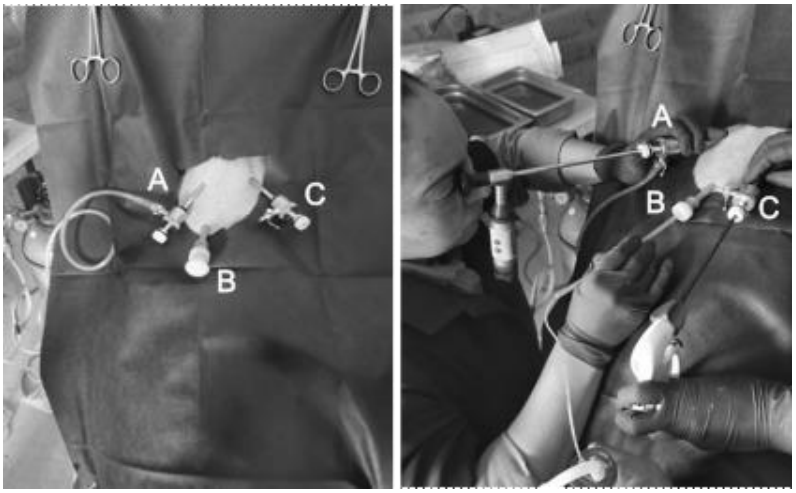

Figure 8. Position of the trocars, endoscope, forceps and suction mandrel. A) Sleeve in which the endoscope is placed. B) Sleeve in which the suction mandrel is placed. C) Sleeve in which the atraumatic forceps is placed to hold the ovary

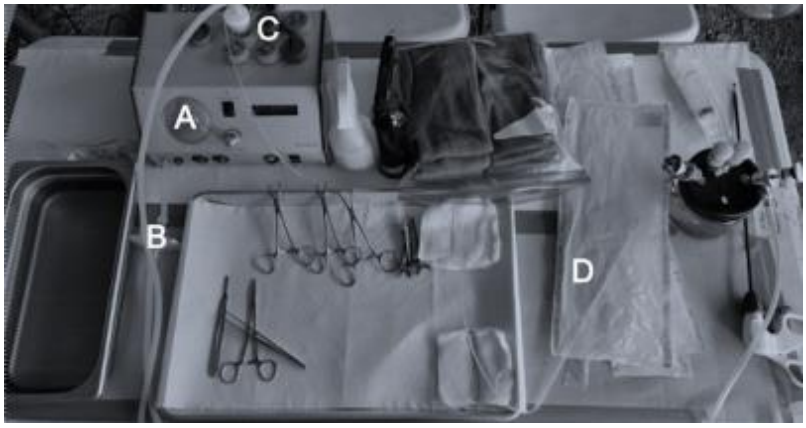

Figure 9. Suction system. A) Suction pump. B) Vacuum hose and filter. C) Collection tube. D) Suction mandrel

INTRAFOLLICULAR OOCYTE TRANSFER (IFOT)

Background

Although intrafollicular oocyte transfer (IFOT) is not a recent technique, it has been reported since 1985 in primates, in cattle (Fleming *et al*., 1985) and in the nineties in equines (Hinrichs & DiGiorgio, 1991). At present, it is associated with new biotechnologies. This technique consists of the transfer of heterologous oocytes into the preovulatory follicle of a previously inseminated female (Figure 10). This technique was perfected in the 1990s through the use of transvaginal ultrasonography in cattle, equine and human reproduction (Kassens *et al*., 2015). This technique application arises in the case of cattle as an alternative to IVP to produce embryos that have greater cryotolerance (Kassens *et al*., 2015). In addition, it avoids the need for laboratory equipment for IVP (Sprícigo *et al*., 2016). With this technique, incubators are replaced by the recipient's uterus and the embryos are obtained using the transcervical uterine lavage technique used in traditional protocols for collecting embryos produced *in vivo*. With IFOT, embryos had been successfully generated in cattle and horses, but it was not until 2015 when the first offspring born from embryos generated by this technique were reported, in which oocytes obtained from trace ovaries and matured *in vitro* were used (Kassens *et al*., 2015). Currently, there are more works where live offspring were obtained by IFOT from immature oocytes (Sprícigo *et al*., 2016; Hoelker *et al*., 2017).

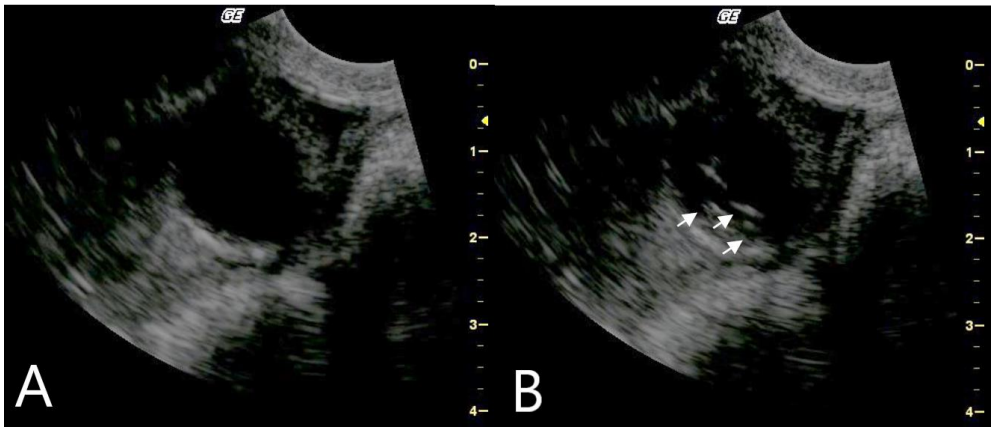

Figure 10. Confirmation of IFOT. A) Preovulatory follicle before IFOT; B) Preovulatory follicle after IFOT, arrows indicate the presence of OCCs (Kassens *et al*., 2015)

This technique has also been used for the *in vivo* production of ovine embryos by IFOT (Falchi *et al*., 2022). IFOT would be complemented with LOPU for the collection of oocytes from donors with high genetic value, which would be transferred to recipients (commercial animals) for *in vivo* embryo production, so that adverse effects such as adhesions would not occur in the donors and this would extend their useful life. It is important to emphasize that, although this technology is promising, the results obtained with traditional IVP are not yet achieved (Kassens *et al*., 2015; Sprícigo *et al*., 2016; Hoelker *et al*., 2017; Falchi *et al*., 2022).

CONCLUSIONS

Ovarian follicular aspiration in ruminants is constantly improving, which facilitates its application. There are still aspects to improve, such as increasing the quality of the oocytes, increasing the useful life of the donors, increasing the number of oocytes collected, as well as the selection of the oocyte donors. Currently, research continues in these areas and everything indicates that in the coming years follicular ovarian aspiration with its variants will be consolidated as effective reproductive tools as their predecessor techniques did.

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Errata Erratum

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