In vitro fertilization in small ruminants: a review

Fecundación *in vitro* en pequeños rumiantes: una revisión

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This document is a bibliographic review on in vitro fertilization (IVF) in small ruminants, describing the different procedures for in vitro embryo production in sheep and goats, as well as the factors that can influence the results of IVF. This research contributes to the strengthening of theoretical knowledge on the in vitro fertilization technique applied in small ruminants. In order to use this reproductive biotechnology, it is necessary to have an equipped laboratory and to have knowledge about the physiology of reproduction in small ruminants and about the elaboration of the necessary means for the production of embryos.

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Abstract

In vitro fertilization is a biotechnology that helps to increase animal genetic improvement by producing embryos from donors with superior production characteristics. It also shortens the generation interval by favouring the obtaining of oocytes from prepubertal animals. In addition, it is a technique that can be used to activate reproductive function in animals that do not respond to superovulation treatments, sick animals and those that are sent to slaughterhouse, but that have great genetic potential. To perform this procedure, the oocytes can be obtained from the living animal by laparoscopic ovum pick-up (LOPU) or collected either post-mortem from slaughtered females. Subsequently, the oocytes that will continue the *in vitro* maturation process are selected, the *in vitro* fertilization itself and finally the *in vitro* culture of the zygotes obtained.

***In vitro* fertilization in small ruminants: a review**		
Objectives	**Methodology**	**Contribution**
To describe the procedures required for *in vitro* production of embryos in small ruminants.	A literature search was conducted on the procedures for *in vitro* embryo production, from the recovery of oocyte directly from the follicles to zygote culture and the factors that influence the success of *in vitro* fertilization (IVF).	This review provides the necessary information to perform IVF in sheep and goats. It is a document that can serve as a guide to implement this reproductive biotechnology in animal reproduction laboratories.

Oocytes, Embryos, Reproduction

Resumen

La fecundación *in vitro* es una biotecnología que ayuda a incrementar la mejora genética animal al producir embriones a partir de donantes superiores en sus diferentes características productivas. También acorta el intervalo generacional al favorecer la obtención de ovocitos de animales prepúberes. Además, es una técnica que se puede utilizar para activar la función genésica en animales que no responden a los tratamientos de superovulación, animales enfermos y los que son enviados a rastro, pero que tienen un gran potencial genético. Para llevar a cabo este procedimiento, los ovocitos pueden obtenerse en el animal vivo mediante laparoscopía o pueden obtenerse a partir de ovarios de hembras sacrificadas en el rastro, posteriormente se debe realizar la selección de los ovocitos que continuarán el proceso de maduración *in vitro*, la fecundación *in vitro* propiamente dicha y finalmente el cultivo *in vitro* de los cigotos obtenidos.

Fecundación *in vitro* en pequeños rumiantes: una revisión		
Objetivo	**Metodología**	**Contribución**
Describir los procedimientos necesarios para la producción *in vitro* de embriones en pequeños rumiantes.	Se realizó una búsqueda bibliográfica sobre los procedimientos para la producción *in vitro* de embriones, desde la recuperación de los ovocitos hasta el cultivo de los cigotos y los factores que influyen en el éxito de la fecundación *in vitro* (FIV).	Esta revisión aporta la información necesaria para realizar la FIV en ovinos y caprinos. Es un documento que puede servir de guía para implementar esta biotecnología reproductiva en los laboratorios de reproducción animal.

Ovocitos, embriones, reproducción

Introduction

In vitro fertilization (IVF) represents one of the most important reproductive technologies in North and Latin America. During the last decade its use has significantly progressed worldwide (Martínez, 2022).

The reproductive efficiency of small ruminants depends on applied biotechnologies, as they play a key role in animal reproduction, making it possible to increase production rates through genetic improvement programmes. Reproduction in sheep and goats is important both for the production of lamb for slaughter and wool and for the production of breeding stock. In order to achieve the above mentioned production goals with high profitability, it is important to achieve significant reproductive efficiency. Currently, IVF has been proven to help increase this efficiency in small ruminant reproduction, leading to higher economic results in sheep production units. IVF has proven to have a great positive impact as a technique for obtaining a high number of useful embryos in sheep and goats, achieving good results both commercially and scientifically. IVF has made it possible to make the most of females in different reproductive stages, such as long-lived, infertile, pre-pubertal, anestrus, sick and even dead females, as they are oocyte donors for *in vitro* fertilization (Cuéllar, 2020).

IVF has demonstrated valuable advantages, such as: offspring with high genetic quality, good quality embryos at a low cost, increased production rate, as well as benefiting females with infertility problems, structural or functional alterations in the genital tract (Herradón *et al*., 2007). IVF reduces the dependence on hormonal treatments that are necessary for superovulation in the case of multiple ovulation production, which reduces costs and mitigates the possible side effects associated with hormonal therapies used in females. In addition, in *vitro* maturation of oocytes eliminates the need for the donor animal to undergo superovulation, which can sometimes lead to poor responses and lower embryo quality (Sharma *et al*., 2024). It is worth mentioning that IVF increases efficiency in selection programmes (Herradón *et al*., 2007) and reduces the risk of transmission of many pathogens (Menchaca, 2023).

IVF involves fertilising an artificially matured oocyte with cryopreserved sperm under controlled conditions. Oocytes are usually obtained by different collection methods, such as follicular aspiration or slicing. IVF has proven to be a useful tool in sheep and goat reproduction, producing a large number of embryos synchronised to a specific stage of development. It is important to know in detail this novel assisted reproduction technique to appreciate the value and impact opportunities that can be generated at a professional and/or business level, as IVF creates alternatives for the storage of genetic resources increasing reproductive efficiency and generating economic benefits (Hernández-Marín *et al.*, 2018). Therefore, this review will detail the procedures to perform IVF in small ruminants.

Oocyte collection methods

Oocyte collection techniques can be performed in two ways: by the follicular aspiration method or the slicing method.

Oocyte collection by follicular aspiration

The follicular aspiration technique involves the aspiration of follicles between 2 to 5 mm in diameter with the help of previously sedated ewes. This procedure consists of making three holes in the caudal part of the female's abdomen, previously disinfected. The holes are made with the help of a trocar, in the left hole the endoscope is introduced, in the central hole a probe with a needle is fixed, the probe is usually approximately 1 cm long to be able to carry out the aspiration of follicles with a diameter greater than 2 mm, the external part of the probe is connected to a tube containing the aspiration medium and the other end is connected to the aspiration pump, while the clamp that allows immobilizing and fixing the ovary is placed on the right side at the time of the aspiration. At the end of the aspiration process, the ovary is irrigated with saline and heparin to prevent the formation of adhesions (Guerrero, 2020).

Oocyte collection by the cutting or slicing technique

While the slicing technique is carried out post mortem. Oocytes are collected and brought to the laboratory in physiological saline, some authors note that antibiotics such as penicillin or gentamicin may be added.

The transport temperature should be considered, the recommended temperature is 30°C, since, if the temperature is low, the *in vitro* maturation capacity is compromised. The cutting technique is carried out with the aid of a sterile scalpel, making several transverse and longitudinal cuts along the surface of the ovary. These cuts guarantee the rupture of the follicles to obtain the follicular fluid, and finally the ovary is washed to obtain the collection of the cumulus-oocyte complex (Martínez, 2013).

Oocyte selection

When selecting oocytes, it is important to take into account three morphological criteria, which are: oocyte diameter, cytoplasmic appearance and the morphological characteristics of the cumulus surrounding the oocyte.

This stage is important because the selection of high quality oocytes will allow better results in *in vitro* fertilization, therefore several methodologies have been considered to classify and evaluate the oocyte quality, based on the critical analysis of the oocytes according to the morphological aspects already mentioned. The classification of oocyte quality can be done in four categories (Mamani, 2017):

- Quality 1 or quality A: These are those oocytes where its cytoplasm is homogeneous and its granulations are fine. It comes to present a large series of compact layers of cumulus cells and is considered an oocyte of excellent quality.
- Quality 2 or quality B: These oocytes have a homogeneous cytoplasm, but small areas of irregular pigmentation can be seen, while their cumulus is compact, but smaller in size with approximately five complete layers, despite this it is considered a good quality oocyte.
- Quality 3 or quality C: They are considered irregular as they present a complete but vacuolised cytoplasm, the zona pellucida (ZP) is covered with approximately three layers of cumulus cells, presenting smaller bare areas.
- Quality 4 or quality D: They have a completely heterogeneous pigmented cytoplasm, their cumulus is partially absent or expanded, they are considered bad oocytes of no quality.

Thanks to this classification, it is possible to recognise that A and B quality oocytes are suitable for use in the *in vitro* maturation process for subsequent fertilization.

It should be noted that the selection of oocytes with a complete cumulus is of great importance for the maturity of the oocytes. This cumulus connects with the somatic cells through cytoplasmic extensions of the granulosa cells, allowing the entry of amino acids and other nutrients that will help to complete the maturity of the oocyte. It has been shown that only oocytes with a compact and dense cumulus are able to complete their development. Likewise, the ability of oocytes to reach maturation *in vitro* depends not only on the size of the follicle or the stage of the oestrous cycle, but also on the presence of a complete cytoplasm, which is considered important to complete maturation. However, the size of the follicles from which the follicles are extracted is still an important point to consider (Jácome *et al*., 2014; Paramio & Izquierdo, 2016).

Factors affecting oocyte quality

Many of the factors affecting oocyte quality are often specific conditions that relate to the environment in which oocytes mature. These conditions limit their ability to be fertilised and usually include factors such as osmolarity, pH, temperature and the time with which the oocytes are transported, as times longer than five hours often affect and compromise the oocyte's ability to mature; culture volume and incubation time are also factors that play an important role in obtaining optimal results in oocyte quality. The oocyte selection stage is also important for proper oocyte development. It is important to know the characteristics of the cytoplasm, the nuclear status, the morphological characteristics of the corona radiata and the expansion of the cumulus cells, as these are conditions that determine the oocyte's maturation capacity (Perea *et al*., 2017).

It has also been pointed out that the presence of corpora lutea or cystic follicles in the ovaries can be a problem for obtaining good results, as they alter the quality of the oocyte, therefore, it is recommended that they are not used in selection programmes (Hernández *et al.*, 2018).

In vitro oocyte maturation

In vitro maturation (IVM) is an important step for successful *in vitro* fertilization. Its results have been of great relevance for reproduction, as it has been able to meet needs and solve existing problems in livestock production. IVM allows the result of potentially good gametes to achieve optimal results in *in vitro* fertilization (IVF). Moreover, IVM is a useful and important tool in the study of oocyte physiology. IVM is the process following oocyte collection and is considered the stage at which the female gametes become capable of being fertilized. The gametes complete their development during IVM by reaching the Metaphase II stage, where they fully mature upon completion of nuclear and cytoplasmic competence.

The success of IVF is highly dependent on IVM, which is why this stage is crucial, as the efficiency of IVM can be compromised by several factors such as: incorrect oocyte collection, transport and temperature, among many others. IVM consists of reaching a certain number of oocytes selected by a sorting method that will allow the selection of oocytes of excellent quality (Fernández *et al*., 2007b).

The IVM process can be carried out in different ways. In the case of obtaining oocytes from sacrificed females, the ovaries are transported to the laboratory in physiological saline solution at a temperature of 35 °C. They are then washed again with saline at a temperature of 37 °C. The oocytes are then collected with the help of a sterile scalpel blade, cutting the follicles which can have a diameter of between 2 to 6 mm. Once the follicle is cut, it is washed with TCM-199 maturation medium supplemented with heparin, gentamicin and HEPES, which is a cell culture medium that helps to buffer the physiological pH. After this, the follicular fluid together with the washing medium is placed in a Petri dish and left to stand for 15 minutes so that the cells settle to the bottom of the dish. After 15 minutes, the search for cumulus-oocyte complex (COCs) is started using an inverted microscope with a 4X objective. Oocytes with three or more layers of cumulus cells and homogeneous cytoplasm are selected (Cuéllar, 2020).

The commonly used maturation medium is TCM 199, which can be supplemented with pyruvate, glucose, hormones (FSH, LH, 17β-estradiol), complex fluids such as fetal bovine serum (FBS), heat-treated serum or follicular fluid recovered from healthy non- atretic follicles, as well as antibiotics such as penicillin and streptomycin (Paramio & Izquierdo, 2014). These culture media must be sterilized through filters and with the help of Millipore pores, which are equilibrated before use in boxes, under a humid atmosphere and a temperature of 38.5 °C for approximately 22 hours. However, in the case of goat oocytes, it has been recommended to use goat serum as it has been shown to correctly promote nuclear maturation and the acquisition of competence at the meiotic level, while in the case of sheep oocytes, SFB is recommended (Andino, 2014). IVM of sheep and goat oocytes is performed in groups of 25 or 50 oocytes and incubated at 38-39 °C in humidified atmosphere of 5% CO2 in air for 24 to 27 hours (Paramio & Izquierdo, 2014).

Maturation media

One of the most important factors limiting efficiency in IVF programmes is IVM, since after careful selection of oocytes, only a little more than a third of oocytes reach full cytoplasmic maturation. The commonly used maturation medium is TCM-199, which may be supplemented with serum of animal origin or derivatives thereof. In this respect, this maturation medium has been designed for somatic cell culture and is considered one of the most widely used media in IVM processes in sheep and goats. TCM-199 is composed of different components such as: hypoxanthine, phosphates and glucose (Ugalde *et al*., 2021).

In sheep, the maturation medium HECM-6 has been used, obtaining a maturation rate of 65%. Likewise, there are other treatments that have been used in maturation programmes such as: SFB (Soberano, 2011) or Synthetic Oviductal Fluid (SOF) (Herrick *et al.*, 2004, Shabankareh *et al.*, 2012).

In vitro fertilization

In vitro fertilization (IVF) is a procedure that involves the interaction and union of gametes, and the subsequent activation of the oocyte to produce a zygote. IVF is defined as the process in which the previously matured oocyte is penetrated by a capacitated spermatozoon outside the genital tract of the female, imitating the interaction of the female gamete with the male, forming pronuclei and carrying out syngamy under controlled conditions (Cuéllar, 2020).

The penetration of the sperm into the zona pellucida of the oocyte occurs within 5 to 15 minutes, with the acrosome reaction occurring before or after the sperm head attaches to the glycoprotein receptors of the zona pellucida. This penetration is important to achieve a correct fusion of the gametes, this penetration is mediated by enzymes that allow the sperm to move into the oocyte (García *et al.*, 2017).

The IVF process is complex, allowing in *vitro* matured oocytes to be incubated with viable spermatozoa in a medium supplemented with energy sources such as lactate, pyruvate and serum albumin. This requires adjustments to heparin concentrations and total sperm count for each individual ejaculate. In IVF, matured oocytes are co-cultured with spermatozoa in specific media and under a controlled environment with culture ovens for 5 to 24 hours, depending on the protocol, sperm concentration and quality of the semen used (Fernández *et al.*, 2010).

The IVF process can be carried out as follows: once IVM has been completed, the oocytes are denuded using a pipette and a constant flow at a pressure of 180 µl. Once the oocytes are denuded, they must be washed twice in order to be placed in a culture medium (Ugalde *et al.*, 2021). The most commonly used medium in sheep is SOF and in goats is Tyrode′s Albumin Lactate Pyruvate (TALP), the medium must be sterilized and equilibrated in an incubator at a temperature of 38.5 °C. At the end of washing, denuded oocytes should be placed in an incubator in 50-100 µl drops of SOF or TALP to be fertilized by spermatozoa at a final concentration of $1x10^6$/ml (Cánovas & Coy, 2008; Paramio & Izquierdo, 2014; Ugalde *et al.*, 2021). Fusion depends on successful nuclear and cytoplasmic maturation of the oocytes. It has been shown that oocytes reach penetration by cryopreserved sperm within three hours of initiating fusion (Cánovas & Coy, 2008).

Sperm capacitation

Sperm capacitation is a process in which sperm undergo changes such as: protein phosphorylation, removal of cholesterol from the plasma membrane and elevation of intracellular calcium. With this capacitation, the sperm gain a vigorous flagellar movement pattern, known as hyperactivation.

Once capacitated, the sperm will be able to penetrate the zona pellucida of the oocyte to successfully fertilize it (Cerdo, 2019).

This process aims to promote the structural and biochemical changes that the sperm undergo. It is an important process for successful IVF. In capacitation, the sperm undergoes the acrosomal reaction that occurs at the plasma membrane level, which facilitates the penetration of the oocyte walls (Gimeno *et al.*, 2017).

Sperm capacitation can be understood as the phases in which the acrosome reaction proceeds, promoting alterations in motility patterns. This capacitation can be achieved by exposing the spermatozoa to concentrations of caffeine and/or heparin, substances that will help stimulate the capacitation process in the spermatozoa, preparing them for interaction and fertilization of the ovum (Jácome *et al.*, 2014).

Gamete fusion

Gamete fusion concludes the IVF process. This stage involves mechanisms of cell recognition and interaction of both gametes. The interaction is carried out from an extensive system of receptors that complement each other, these receptors prove to be a key part in the recognition of the gametes. The zona pellucida undergoes a change where it expands and once the acrosome reaction is initiated, the sperm head is attached with the help of receptors that act as binding proteins on the zona pellucida, subsequently enzymes are released that help in the motility of the sperm to penetrate into the oocyte (Hafez & Hafez, 2000).

Oocyte activation

After gamete fusion, oocyte activation is triggered, which consists of a succession of cellular transformations that are induced by the spermatozoon, thus preparing the oocyte for the transformation of the zygote. After gamete fusion, the release of an oocyte activating factor begins, this occurs in the ooplasm and contributes to induce events of the activation cascade (Castañeda, 2009).

When the sperm penetrates the oocyte, an intracellular calcium release occurs and persists for 3 to 4 hours, causing a reaction in the cortical granules in the oocyte, which releases enzymes that alter the conformation of the zona pellucida. This alteration causes a decrease in the affinity of the spermatozoa for the oocyte, thereby blocking the entry of additional spermatozoa, which is important to prevent polyspermy (Olivera *et al*., 2006).

When the process of oocyte activation begins, the cortical granules begin to line up under the plasma membrane, thus with the activation of the oocyte the membrane fuses and the process of exocytosis begins (De Paola, 2016).

In this process, there is also a series of signalling pathways that complete the second meiotic division in the oocyte and with this the expulsion of the second polar body (Cerdo, 2019).

Chemical activation of the oocyte is carried out by culturing in a medium, e.g. SOF-HEPES containing Ca for approximately 4 minutes at a temperature of 39 °C and then rapidly cultured with another medium in 100 µl drops, e.g. SOF-IVC medium supplemented with bovine serum albumin (BSA) and other compounds for five hours in a humid atmosphere with 5% CO2 in the air at a temperature of 38.5 °C (Benavides, 2012).

In vitro embryo culture

In vitro embryo culture is carried out in species of zootechnical interest, being a biotechnology that plays a role in the productive improvement of herds. This stage comprises a sequential process of three important events carried out under controlled conditions in the laboratory: *in vitro* maturation , *in vitro* fertilization of matured oocytes and *in vitro* culture of fertilized oocytes (Fernández *et al.*, 2007a).

The process begins when the embryos have more than four cells resulting from *in vitro* fertilization, at which point they are removed from the maturation medium to continue their cell division process in a special embryo culture medium, where they will complete the division of eight, sixteen and thirty-two cells to become morulae and blastocysts. Upon reaching these stages, the embryos are transferred to recipient females in two ways: fresh or frozen. In this process, energy sources such as glucose and some amino acids can be used. *In vitro* embryo culture is usually the longest stage of the whole process, where the overall efficiency of the programme is determined and the quality of the process is demonstrated by the embryos obtained (Ugalde *et al*., 2021).

After IVM, only approximately 90% of the immature oocytes that are cultured reach metaphase II and expel the first polar body. Of the total number of oocytes, 80% reach fertilization and begin to divide. However, only 25-40% reach the blastocyst stage. Somatic cell culture or somatic cell conditioned media supplemented with serum is often used in order to avoid developmental blockage, which leads to embryo death. However, it has also been shown that the addition of serum to the culture media is the main factor responsible for the development of fetal over-volume syndrome, as well as affecting the resistance to cryopreservation of the embryos obtained, causing low resistance (Tarazona *et al.*, 2010).

Culture conditions

Culture conditions are biophysical parameters and organic elements that must be controlled in the culture media, these parameters are usually: osmolarity, pH, most mammalian embryos cultured *in vitro* develop a neutral or slightly alkaline pH and bovine or ovine oviductal fluid which are characterized by minimal levels of Na and high levels of K (Salgado & Lopera, 2020).

As for the organic elements that must be taken into account are: the source of energy, the most commonly used in culture media are lactate, pyruvate and glucose. The source of protein should also be taken into account, such as amino acids, which are relatively important elements as they are involved in the regulation of embryo development (Mucci *et al.*, 2006).

Oxygen tension is one of the culture conditions that must also be taken into account, most mammalian embryos in the reproductive tract handle an oxygen tension of 3.5 to 8%, therefore, embryos cultured *in vitro* must use a similar oxygen tension.

This culture condition has several benefits, some of which are that it improves the rate of embryo production, increases cell number and reduces the production of free radicals. The use of serum free amino acids in *in vitro* culture media increases embryo development, due to its antioxidant action and the control it exerts on pH. It should be taken into account that the use of amino acids should be replaced every three days due to the high concentrations of ammonium in the medium (Benavides *et al*., 2015).

Culture media

Culture media are intended to increase metabolic efficiency; therefore, these media must be an excellent source of energy. In sheep, the medium known as synthetic oviductal fluid (SOF), which was developed by Tervit, comes into use. Culture media are generally solutions supplemented with components such as magnesium, calcium, phosphates, sulphates and bicarbonate. As well as water is one of the main components providing a higher proportion in the formulation of various types of culture media (Ortega *et al.*, 2022).

Protein sources are also often important in culture media, amino acids are important elements in regulating embryo development (Ramírez, 2020).

Evaluation of *in vitro* fertilization

In vitro fertilization can be assessed by observing the segmentation rate at 18 and 48 hours post-fertilization; the morula and blastocyst stage at 6 to 8 days or it can be assessed after a 20-hour incubation period. The oocytes are usually washed with 3% sodium citrate, observed under a microscope, denuded and fixed with the aid of an ethanol-acetic acid mixture over a period of about 24 hours at a temperature of 4°C. After this, they are stained with 1.1 % acetate-orcein and classified as follows:

- Normally penetrated: these are those that present two pronuclei in the cytoplasm of the zygote, one female and one male; certain morphological characteristics may also appear, such as a sperm tail or the decondensed head of the spermatozoon.
- Asynchronous: these are oocytes that are usually penetrated by only one sperm. One of their characteristics is that an alteration in the development of the two pronuclei can be observed.
- The third group are oocytes that remain arrested in the telophase II stage, although they are usually activated oocytes.
- The last group are the polyspermic zygotes, whose characteristics are very peculiar as they usually have more than two pronuclei and two polar corpuscles. Another of their characteristics is that more than two tails or heads of spermatozoa can be observed decondensing (Báez *et al*., 2010).

***In vitro* fertilization abnormalities**

In many cases, oocytes develop abnormalities mainly in the IVM process. Sometimes these abnormalities are due to a lack of maturity in the oocyte cytoplasm or due to cytoplasmic deficiencies that manifest themselves in the formation of pronuclei. During maturation *in vitro*, nuclear processes, germinative vesicle rupture and polar corpuscle formation appear to proceed normally, however, it should be noted that fertilization, division and early embryo development are often unsuccessful due to developmental disruption resulting from incomplete cytoplasmic maturation, which is why some authors mention that oocytes mature better *in vivo* than *in vitro*. However, it is said that certain hormonal or follicular factors are required to improve cytoplasmic maturation and thus have results with successful fertilization rates and embryo development (Guzmán *et al.*, 2016).

The ability of the zona pellucida to be penetrated may be compromised, if maturation conditions are not adequate, *in vitro* matured oocytes may not acquire full competence to be fertilized (Salgado & Lopera, 2020). It has been observed that the formation of the pronucleus of oocytes matured *in vitro* is not only minimal compared to oocytes that mature *in vivo*, but that the formation of both pronuclei becomes asynchronous. These results lead to the conclusion that in *vitro* matured oocytes may lack the male pronucleus growth factor, resulting in the formation of only one or neither pronucleus. Asynchrony of pronucleus formation is defined as a delay in the development of the male pronucleus after the normal formation of the female pronucleus following fertilization. This presence of a single pronucleus in oocytes may be related to a probable deficiency of key molecules that help regulate the blockage of meiotic division and thus become involved in the control of later stages of cell cycle progression in the mature oocyte (Martínez, 2018).

On the other hand, polyspermy is a very common anomaly that affects mature oocytes fertilized *in vitro*, accounting for 80% of the most frequent fertilization problems. Some authors have shown that a large proportion of the oocytes with this problem are usually cases of dyspermia. Although the exact process involved in blocking polyspermy is not yet known, a wide variety of causes have been explained, mainly involving cortical granules (Martínez, 2018).

Factors influencing the success of *in vitro* fertilization

There are a large number of factors that drastically influence the variability of *in vitro* fertilization, these factors can be technical, biological or in some cases genetic. Some factors that come into consideration during the IVF process are the inseminant concentration, the treatments given prior to fertilization and the sperm source. On the other hand, technical factors such as the skill, technique and experience with which the technical team carries out the stimulation and oocyte aspiration, as well as the entire remaining process, must be taken into account (Salgado & Lopera, 2020). There are also other factors that influence the success rates of IVF, for example; the age of the female, as pre-pubertal ewes or goats respond better than adults. Meanwhile, the selection and classification of oocytes is also critical for IVF success, because IVF is highly dependent on IVM (Lopera, 2011; Martínez, 2018).

Some seminal alterations can also hinder fertilization, which is why it is essential that the number, motility and morphology of the spermatozoa are appropriate. On the other hand, the conditions under which the Cumulus-Oocyte Complexes (COC's) are transported can also affect the competence of IVF, as well as the presence of corpora lutea and cystic follicles in the ovary at the time of oocyte aspiration, which is why it is not recommended to use this type of ovaries that compromise oocyte fertilization. It is important to take into account the characteristics of the ewes from which the ovaries come, because if they are not in the right conditions they can affect the oocyte quality and thus compromise the capacity for *in vitro* development. Environmental factors, such as the physical conditions of the medium in which oocyte maturation takes place (pH, osmolarity and ionic composition of the medium; temperature and CO_2 and O_2 tension of the incubator; culture volume and incubation time), as well as the maturation medium can also influence the oocytes, compromising oocyte quality. For this reason, each stage of *in vitro* embryo production must be carried out carefully, to decrease the likelihood of IVF being affected (Martínez, 2018).

Conclusions

This research contributes to the strengthening of theoretical knowledge on the *in vitro* fertilization technique applied in small ruminants. *In vitro* fertilization is gaining momentum in these species, providing valuable opportunities for reproduction and genetic improvement, as well as having a positive impact on scientific progress, helping to expand knowledge about the stages involved in the complex phenomenon of reproduction.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest.

Author contribution

Sagastume-Dulce: Did the research and writing of the manuscript.

Tabarez-Abigail: Contributed to the research idea, revision of the research and editing of the manuscript,

G*arcez-Nora and Alarcón-Marco:* Did the revision and editing of the manuscript.

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Background

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Basics

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