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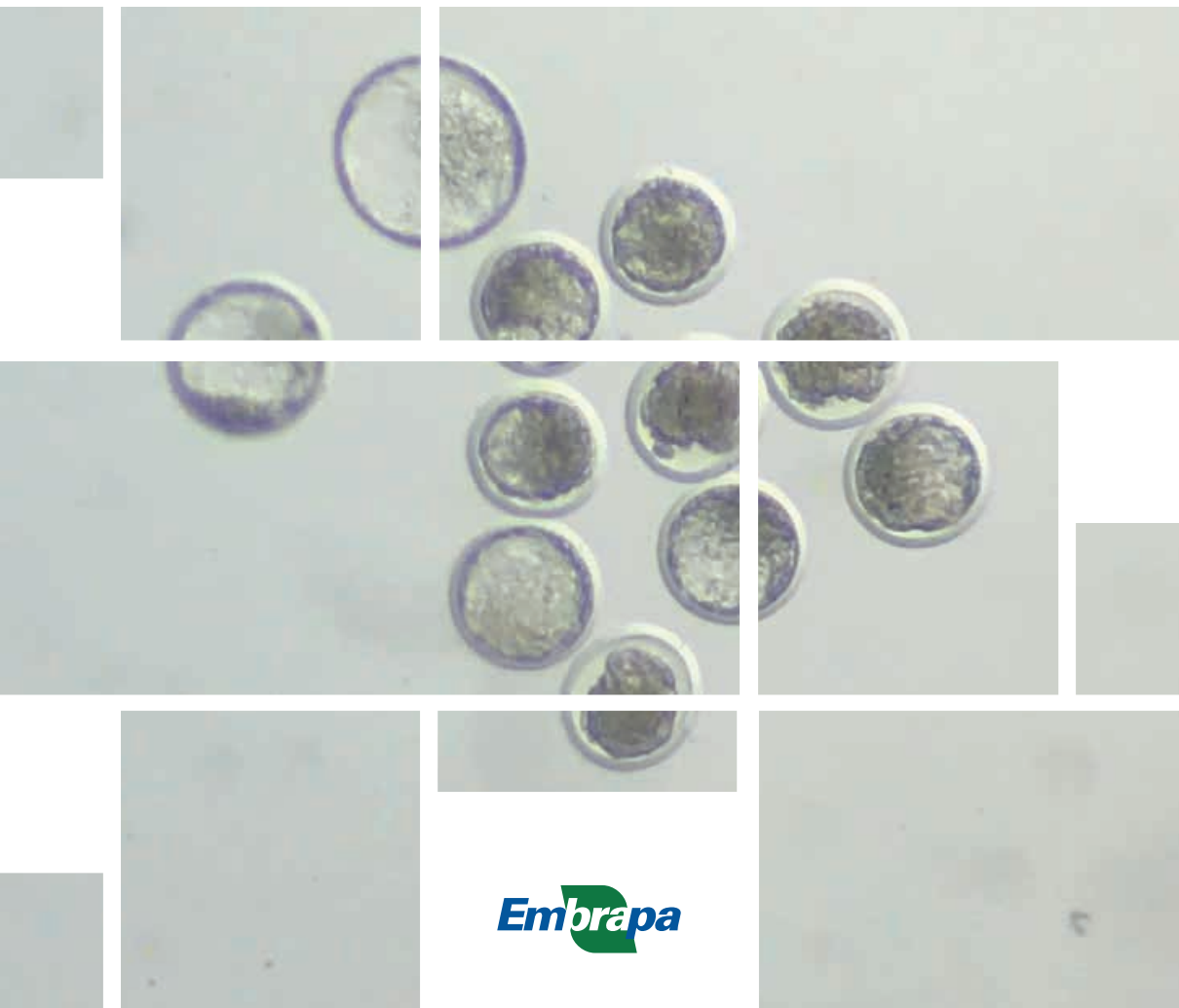

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Presentation

Brazil is known for its enormous natural animal resources, typically associated with a large diversion of biomes. Although they do not originate in the country, the sheep and goats that were incorporated by settlers after the discovery became the so-called locally adapted animals. This adaptation obeyed the environmental challenges imposed, being more severe in the semi-arid zone - Caatinga biome. Similar to the African savannah, important and unique genotypes of small ruminants live in the Caatinga. Most of them are under some degree of extinction risk mainly because there is no economic interest and/or indiscriminate reproduction with commercially exploited sheep and goats around the world, such as Dorper and Boer, respectively. These specimens were included in the Brazilian Germoplasma Bank.

Many genetic conservation strategies for these offspring include maintaining conservation units for a few dozen females and a few males, DNA, semen and embryos. The embryo biobank is the most viable and fastest strategy for preserving genuine specimens and rebuilding the herd, being the best option for preserving mammals in a situation of extinction. Initially, the Brazilian Bank of Embryos of Sheep and Goats was supported by the surgical recovery of embryos. Over time, after great Brazilian efforts and increases in the efficiency of non-surgical embryo recovery (NSER), the embryo bank became more pronouncedly composed of embryos of NSER origin.

This document will take the reader through the history of the Brazilian Embryo Bank of Sheep and Goats with important information about the breeds included and techniques for preserving them.

Marco Aurélio Delmondes Bomfim
Head of Embrapa Goats & Sheep

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Introduction

Global production of food and agriculture depends on plant, animal, and microbial genetic resources. The important animal species in existence today are a consequence of a domestication process that stretches back almost 12,000 years, while genetic diversity is the result of livestock and controlled reproduction, combined with the effects of natural selection. Although 50,000 species of birds and mammals are known, about 40 species have gone through some domestication process, with fewer than 10 species accounting for more than 90% of global livestock production, such as the FAO (2020). Projections suggest that global meat and milk consumption will continue to increase until 2030, and growth consumption is projected at 1.6% and 1.3% per year, respectively, for meat and milk (Alexandratos; Bruinsma, 2012). As a result, livestock farmers need to find increasingly efficient means to adapt their animal genetic resources, thereby increasing the number of rare and productive breeds at the expense of the total number of breeds. Variation between and within breeds is under pressure as a result of selection, breed replacement, or genetic drift. Many breeds of domestic livestock are threatened or endangered. There are about 8,800 recorded domestic breeds of birds and mammals, of which 7% are extinct and 24% at risk of extinction. Everywhere, it is predicted that about 50 or more breeds will be lost per year, which is approximately one breed per week (FAO, 1998). In addition, many breeds are reduced to numbers that make their future very unsafe if no action is taken to conserve them. Thus, not only is the conservation of genetic diversity crucial to ensure continued availability for adaptation to climate change and future production, but it is also necessary to preserve options for development and use in genetics and animal breeding.

A germplasm biobank is the most appropriate choice for preserving pure breeds for long-term storage in the form of frozen semen or frozen embryos. Semen cryopreservation is a widely used technique in veterinary medicine (Ehmcke; Schlatt, 2008). Embryos can be produced and recovered by means of different techniques (Fonseca et al., 2016). Superovulation is the best means of producing a large number of embryos per donor (Fonseca; Oliveira, 2020) and, along with non-surgical embryo recovery (NSER), a consolidating technique able to efficiently change surgical processes, is now the method of

choice globally. It allows for the prompt restoration of breeds after preserved embryos are transferred to recipient females (Boettcher et al., 2005; Ehling; Niemann, 2000). Once produced and recovered, embryos can be stored in liquid nitrogen for an undefined period after being subjected to the cryopreservation process.

This manuscript deals with the Brazilian Germplasm Bank, with a focus on the techniques of production, recovery, and cryopreservation of sheep and goat embryos aiming at the genetic conservation of locally adapted breeds.

Current situation of sheep and goat genetic resources

Sheep and goats are the most affordable animals in the world and can be accommodated in any type of weather conditions. According to the FAO (2019), the estimated population sizes of domestic sheep and goats are about 1,401 and 1,093 million, respectively. They contribute to human needs by providing meat, milk, fiber, and other resources such as manure, which is used as fertilizer. In addition, small ruminants serve as a very important cash reserve for many small farmers, play an important social and cultural role, and are essential components for achieving sustainable food security.

Due to the growing demand for food of animal origin, farmers in several developing countries have decided to establish breeding programs to improve productivity but without placing sufficient emphasis on preserving general genetic diversity. The efficiency of modern selection methods has successfully increased production; however, many sheep and goat breeds now suffer from inbreeding, with effective population sizes falling below 50 individuals. With the development of these industrial breeds, there has been economic pressure on farmers to abandon their traditional breeds; as a result, many have recently become extinct. The latest of the Food and Agriculture Organization of the United Nations (FAO) survey found that 10.4% and 2.8% of sheep and goat breeds, respectively, have already disappeared (FAO, 2015) (Figure 1), while a further 12.4% of sheep breeds and 13.4% of goat breeds are classified as at risk. However, in absolute terms, the number of breeds at risk is greater among sheep (191 breeds) than among goats (91 breeds).

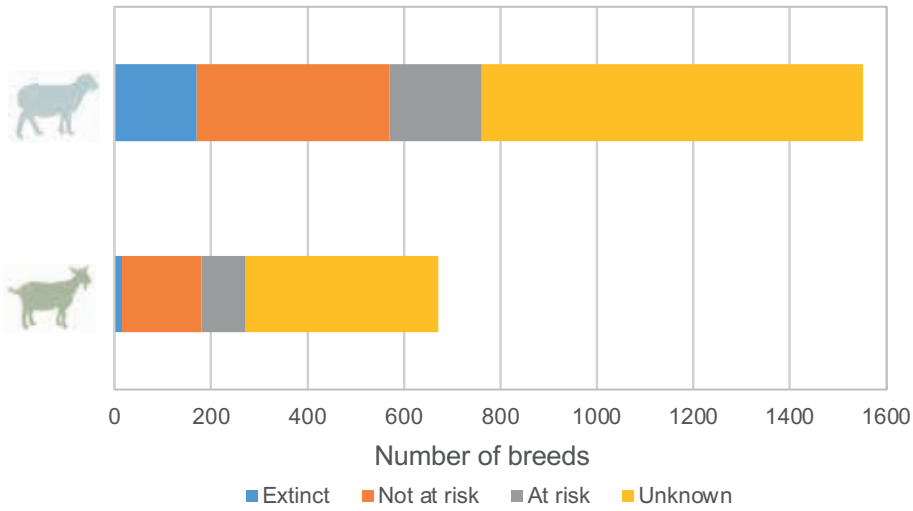


Figure 1. Status of the world's sheep and goat breeds.

Adapted from FAO (2015).

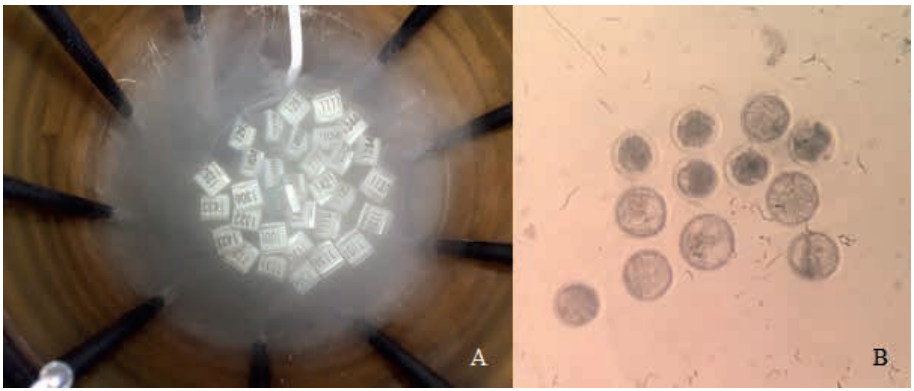
In Brazil, most species of domestic animals were introduced through successive journeys by Portuguese settlers after the European colonization. Several breeds of sheep and goats were developed through the process of natural selection in different environments and climatic conditions, resulting in breeds which are considered locally adapted (Mariante et al., 2003). These breeds are known for their rusticity and adaptability, which gives them important attributes as genetic resources. However, due to their reduced productivity and the need to satisfy growing consumer demand, farmers are tending to replace local breeds with exotic breeds or use indiscriminate cross-breeding (Mariante; Cavalcante, 2006). These two factors are the most important causes of genetic erosion, and it is becoming increasingly urgent to implement sound conservation strategies for these species (FAO, 2007).

Conservation of animal genetic resources

Conservation strategies can be categorized as *in situ* conservation (in which animals are kept within the environments or production systems in which they were developed) or *ex situ* conservation. The latter involves maintenance and breeding of endangered animals in selected areas outside their natural habi-

tat, including germplasm *in vitro* conservation in liquid nitrogen at a temperature of −196°C (cryopreservation). *In situ* conservation mainly involves the active creation of animal populations for food and agricultural production, so that diversity is used optimally in the short term and maintained in the long term.

Cryopreservation is the collection and freezing of semen, eggs, and embryos for future use in animal reproduction or regeneration (Figure 2). For the conservation of animal genetic resources, both semen and embryos have been proposed as the most suitable materials for cryopreservation. Semen can be collected and frozen easily for most species, while embryo collection and freezing are much more expensive and require greater technical capacity. Reconstructing the breed with semen alone can be accomplished through a series of backcrossed generations, but this approach has some limitations, including the fact that 100% of the original breed's genetics can never be recovered (Boettcher et al., 2005). On the other hand, embryo cryopreservation is more expensive, but it allows the conservation of the entire genetic complement so that the regeneration of the breed is faster and cheaper (FAO, 2012).



Photographs: Kleibe de Moraes Silva

Figure 2. Cryopreservation of germplasm. A) Semen (B) Embryos.

To prevent the disappearance of local breeds worldwide, the FAO initiated contacts in 1987 to install regional animal gene banks for developing countries. At the time, Embrapa Genetic Resources & Biotechnolog (Cenargen) was chosen to host the bank that would be responsible for the storage of semen and embryos from breeds of domestic animals at risk of extinction (Mariante; Egito, 2002) (Figure 3). Semen and/or embryos from cattle, hor-

ses, donkeys, goats, sheep, fish, and pigs are stored at the Cenargen Animal Germplasm Bank (CAGB), which presently holds almost 110,000 doses of semen and more than 880 embryos (Alelo Portal, 2021).

Photograph: Melo (2021).

Figure 3. Infrastructure of Embrapa's Genetic Bank, inaugurated in 2014 at Cenargen. A) Building and (B) cryopreservation facilities.

Source: Brazil Agency - EBC (2014).

Most local sheep and goats breeds are at risk, either due to the small number of herds or low genetic variability within and between herds. Some breeds are not yet recognized as breeds by the Brazilian Ministry of Agriculture, Food and Supply (MAPA), most of which are located in the northeast of Brazil (McManus et al., 2014). This region is dominated by long dry seasons, low humidity and precipitation, high temperature and poor vegetation. These animals still have tolerance of or resistance to disease (Ianella et al., 2012) and parasites (Toscano et al., 2019; Haehling et al., 2020), as well as the ability to adapt to the availability of food resources and heat stress (Leite et al., 2018). Several breeds were included in the Embrapa cryopreservation program, especially endangered local breeds, for which the cryopreservation is the favored conservation strategy (Mariante et al., 2009). Currently, 35,982 and 649 doses of sheep and goat semen and embryos, respectively, are stored at CAGB (Alelo Portal, 2021) (Table 1).

Unlike the practices applied to commercial herds, conservation prioritizes individuals and their health. Therefore, and in accordance with the growing concerns about animal wellbeing, Brazil has recently become the worldwide leader in NSER (Fonseca et al., 2019b). Initially, the majority of the stored embryos at CAGB were collected by surgical recovery; however, the less invasive and less stressful method (NSER) of embryo recovery (Santos et al., 2020)

was used for the majority of stored goat and sheep embryos in the Brazilian Biobank (Figure 4).

Table 1. The situation of the Brazilian semen and embryo bank for locally adapted goats and sheep.

Species / Breed	Semen		Embryos	
	Donors	Doses	Donors	Embryos
Goat				
Repartida[1]	3	42	0	0
Nambi[1]	2	628	0	0
Azul[1]	12	1,177	0	0
Canindé	12	2,201	9	35
Gurguéia[1]	0	0	0	0
Marota[1]	27	2,662	0	0
Moxotó	29	3,065	14	98
Sheep				
Barriga Negra[1]	0	0	0	0
Bergamacia	4	1,098	9	28
Cariri	0	0	0	0
Crioula Lanada	37	4,697	0	0
Morada Nova (Red)	43	5,871	16	126
Morada Nova (White)	3	796	0	0
Pantaneira[1]	0	0	0	0
Rabo Largo	3	718	0	0
Santa Inês	42	11,219	31	256
Somalis Brasileira	7	1,808	19	106
Total	224	35,982	98	649

[1] Not an official breed recognized by the Ministry of Agriculture, Livestock and Food Supply (MAPA).

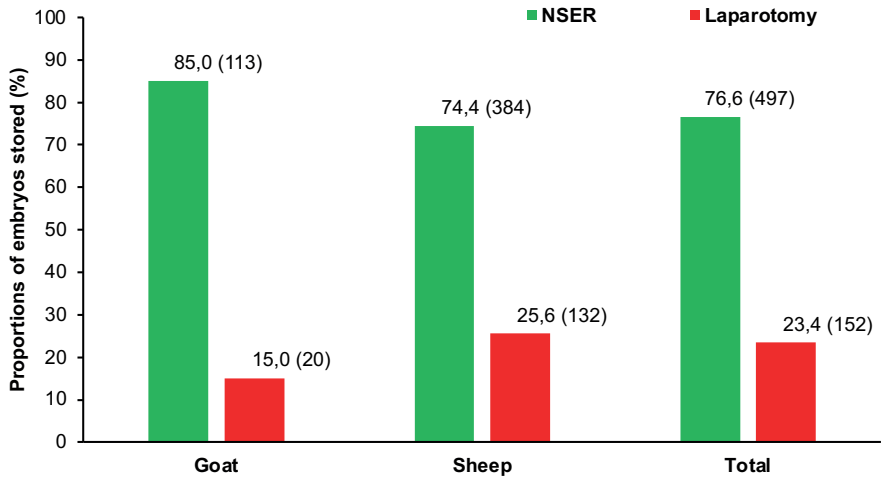


Figure 4. Proportions of stored goat and sheep embryos collected by surgical (laparotomy) and non-surgical embryo recovery (NSER).

Procedures for the collection and cryoconservation of sheep and goat embryos have largely been developed in recent years, and more resources have been spent on development and refinement. As a result, success rates are generally considerably bigger than in previous decades. Several techniques have been reported for collection and cryopreservation, each of which presents different difficulties, types of expertise, success rates, and costs. The following subsections describe the options currently in use in cryobanking.

In vivo embryo production

in spite of new insights for *in vitro* embryo production (IVP), in vivo derived embryo (IVD) production remains the main source of goat and sheep embryo production (Souza-Fabjan et al., 2021). Normally, the embryos of donor goats and sheep are collected six to seven days after estrous onset. There are several means of obtaining IVD embryos, some simple and some more laborious. The first way includes embryos produced from natural estrus (Fonseca et al., 2013), synchronized estrus (Fonseca et al., 2019b), and synchronous estrus-induced (Prellwitz et al., 2019; Figueira et al., 2020c). The second way involves multiple ovulation preparation using various and greater amounts of exogenous gonadotropins: the so-called superovulation.

Natural Estrus

Although the use of natural estrus to supply embryo production is not common, it is possible. Animals under assisted breeding can produce high-quality viable embryos that can be recovered seven days after first natural mating. It has been reported that a donor goat previously subjected to superovulation and embryo recovery was hand-mated one day before embryo collection and again subjected to embryo recovery seven days later, resulting in three blastocysts (Fonseca et al., 2013).

Estrus Synchronization

Estrus synchronization can be carried out by using two doses of prostaglandin F2-α (PGF2- α) analogue (Ex. Cloprostenol) administered 7.0 to 11.5 days apart in goats (Maia et al., 2017; Bonato et al., 2019) and sheep (Carvalho-de-Paula et al., 2020). The use of two doses of cloprostenol seven days apart has produced viable embryos collected non-surgically in Santa Inês sheep (Fonseca et al., 2019b).

Synchronous Estrus Induction

Synchronous estrus induction can be successfully provided by means of a hormonal cocktail with an intravaginal progesterone- or progestagem-releasing device kept in place for from 6.0 to 12.0 days, plus cloprostenol and equine chorionic gonadotropin (eCG) administered 24 h before device removal. This relatively simple estrous cycle control can produce a considerable number of viable embryos in goats (Esteves et al., 2013) and sheep (Figueira et al., 2020c; Arrais et al., 2021).

Superovulation

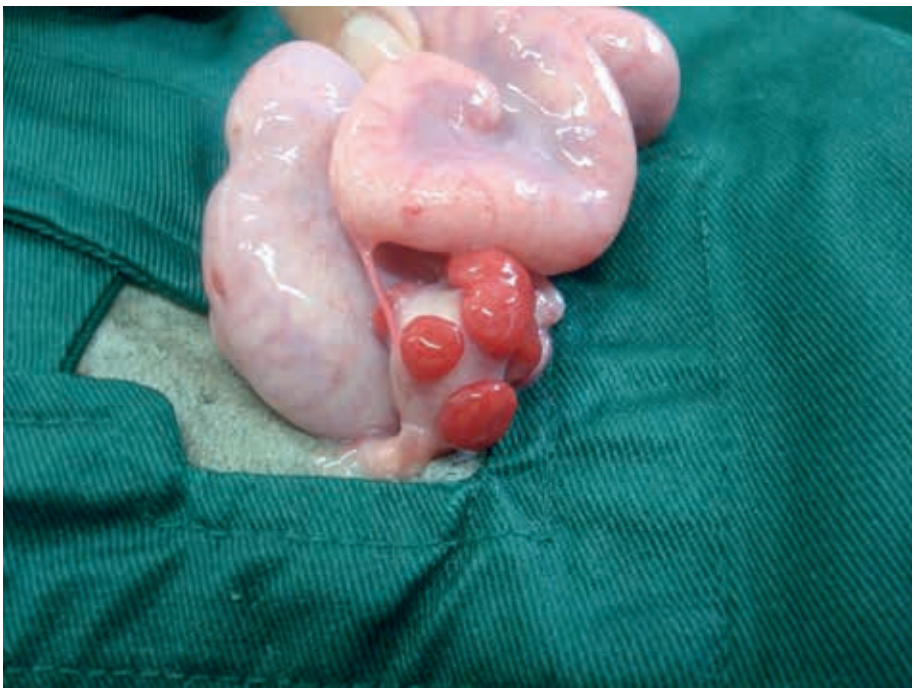
Superovulation is a hormonal preparation used to overcome the normal ovulatory rate of a given female. This technique is based on supplying large amounts of exogenous follicle-stimulating hormone, normally of porcine origin (pFSH), which are strategically administered at the end of the estrus synchronization protocol with cloprostenol in goats (Esteves et al., 2013) or with

intravaginal progesterone devices in goats (Amorim et al., 2011; Fonseca et al., 2013; Maia et al., 2020) and sheep (Figueira et al., 2020b; Figueira et al., 2020a). Two tested protocols for both goats and sheep managed in tropical conditions are suggested (Fonseca; Oliveira, 2020).

Techniques for embryo recovery

Basically, embryos can be recovered by means of the four techniques described below. Some comparisons are given for the three main techniques (Fonseca et al., 2019a).

Laparotomy



Photographs: Jeferson Ferreira da Fonseca

Figure 5. Adherence between ovarian and uterine horn in a sheep subjected to embryo collection by laparotomy two months previously. Despite presenting a good ovarian response to superovulation, this type of adherence may hinder the capture of oocytes by the fimbria during ovulation, resulting in insufficient or non-existent embryo collection from this uterine horn.

Laparotomy is the worldwide technique of choice for embryo recovery in goats and sheep. This technique involves previous food (24 h to 36 h) and water privation (12 to 24 h) prior to laparoscopy evaluation for corpora lutea (CL) count. Normally, donors with superovulatory response ≥4 CL are subjected to general anesthesia before midventral incision anterior to udder insertion is performed. Thereafter, the uterine horns are exteriorized. A Foley catheter number 8 to 10 with an inflatable balloon is inserted near the intercornual ligament while the cranial edge of the uterine horn receives an injection of 15 to 40 mL flushing media recovered by the catheter. Although this technique is recognized as efficient for embryo recovery, like other surgical procedures it is associated with relatively more risk to life related to anesthesia, digesta aspiration, undesirable perforation of abdominal organs and blood vessels, and sequels such as adherences (Figure 5) that decrease embryo recovery efficiency in successive attempts in goats (Lehloenya et al., 2010) and sheep (Üstüner et al., 2014; Pinto et al., 2020).

Laparoscopy

Laparoscopy is also considered a surgical procedure and requires all the prior steps and preparations as laparotomy. Although less invasive than laparotomy, it requires a highly skilled technician and special collection device inserted through the abdominal wall. This device, like the Foley catheter, has an inflatable balloon. It is inserted in the portion similar to Foley catheter after uterus has been fixed. Thus, flushing media are administered into the cranial portion of the uterine horn and removed through the device (McKelvey et al., 1986).

Semi-Transcervical

An experimental semi-transcervical technique has been described for embryo recovery in goats (Moura et al., 2011). This procedure involved surgery and required a technician to introduce one hand through the paralumbar fossa incision, manually ensuring the correct passage and positioning the Foley catheter number 8 to transpose the cervix by the vaginal route. After inflating the Foley balloon, flushing media were injected and recovered as with bovine species. The procedure was performed in 1.5 h per donor and, although the authors considered this to be a promising technique, no later report has been found.

Non-Surgical Embryo Recovery (NSER)

NSER is considered the most promising technique for embryo recovery in goats and sheep (Fonseca et al., 2016; Fonseca et al., 2019a). This technique is now reported to achieve successful embryo recovery regardless of how the animals are prepared after natural estrus (Fonseca et al., 2013), whether estrous synchronization (Fonseca et al., 2019b), induction (Esteves et al., 2013; Figueira et al., 2020c; Arrais et al., 2021), or superovulation (Amorim et al., 2011; Figueira et al., 2020a; Maia et al., 2020). The procedure involves simple anesthesia (Fonseca et al., 2016) and there is no need for food or water fasting (Figueira et al., 2020c; Arrais et al., 2021), with the donor kept in a standing position (Fonseca et al., 2019a). Currently, NSER is preceded by a cervical relaxation protocol after the administration of cloprostenol only (Fonseca et al., 2013; Maia et al., 2020) for goats or a combination of cloprostenol and oxytocin (Dias et al., 2020) or cloprostenol-estradiol benzoate--oxytocin (Figueira et al., 2020a; Oliveira et al., 2020) for sheep, resulting in efficient and sustainable embryo recovery in both goats and sheep.

Cryopreservation techniques

There are two main techniques for the cryopreservation of sheep and goat embryos. The first to be developed, and in more common use worldwide, is based on controlled freezing, going from positive (20 ºC) or negative (-5 ºC) to a stabilizing temperature (-32ºC), at which embryos inside French straws are plunged into liquid nitrogen (N2). The technique requires an electronic freeze machine. The other technique, which is not considered a freezing process, is vitrification, which can be performed using greater amounts and combinations of cryoprotectants in growing concentration baths, finishing with plunging into liquid nitrogen. Embryo cryopreservation using a slow cooling rate and dehydration and rehydration for direct transfer have been successfully tested in goats (Fonseca et al., 2018) and sheep (Figueira et al., 2019; Brair et al., 2020), resulting in sustainable embryo survival and pregnancy rates.

Concluding remarks

Germplasm banks can have multiple purposes. While their primary function is the conservation of genetic resources for use in the medium or long term, a

further common purpose is to provide the possibility of minimizing inbreeding and genetic drift in small managed populations, as is the case for locally adapted breeds. A second potential purpose is to recreate breeds or breeding lines if they are lost as the result of loss of genetic diversity due to the artificial selection process. The Brazilian Embryo Biobank of goats and sheep is seeing ongoing and significant growth, which can be seen as a sustainable and efficient method of biopreservation of locally adapted genomes. The Brazilian Embryo Biobank probably differs from other biobanks because it is based on local experience and effectively proved embryo production, non-surgical recovery, and cryopreservation, all technologies which were developed under Brazilian conditions.

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Goats & Sheeps

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