BIOPRESERVATION AND BIOBANKING
Volume 00, Number 00, 2021

DOI: 10.1089/bio.2021.0041

Transcervical Versus Laparotomy Embryo Recovery: What Strategy Is Best for Embryo Bank Formation in the Canindé Goat Conservation Program?

Joanna M.G. Souza-Fabjan, JMG,[1,i] Ribrio I.T.P. Batista, RITP,[1] Luciana M. Melo, LM,[2] Marcos A.L. Oliveira, MAL,[3] Maiana S. Chaves, MS,[4] Jeferson F. Fonseca, JF,[5,ii] and Vicente J.F. Freitas, VJF[4,iii]

By allowing for the creation of embryo banks, reproductive biotechnologies play an essential role in the preservation of endangered goat breeds' genetic diversity. This study focused on comparing both available embryo collection methods [laparotomy (LAP) vs. nonsurgical embryo recovery (NSER)] in Canindé goats to create an embryo bank for later use in a breed conservation program. Twelve females were superovulated and subjected to either the LAP or NSER technique for embryo recovery. The recovery rate was similar ($p > 0.05$) between NSER (86.8% ± 5.6%) and LAP (92.8% ± 4.0%). Moreover, there were no differences ($p > 0.05$) in the number of structures recovered, the viable embryos, and the freezable embryos per goat, respectively, for NSER (11.7 ± 1.3, 11.2 ± 1.5, and 10.2 ± 1.1) and LAP (10.3 ± 1.0, 8.7 ± 0.7, and 8.0 ± 0.8). Overall, 132 structures were collected out of 151 ovulations (~12.6 ± 1.2 corpora lutea per goat). Finally, the procedure duration time was also similar ($p > 0.05$) for NSER versus LAP, respectively: 32.3 ± 3.3 versus 30.8 ± 3.9 minutes. In conclusion, the NSER method results proved to be similar to the LAP technique in small-sized Canindé goats. It was noticeable, however, that the NSER technique is simpler and provides the possibility for successive procedures with few health risks and sequels for females. This study may hopefully boost *in vivo* embryo production programs in the Canindé breed, facilitating the formation of embryo banks and so assuring the availability of genetic diversity before any decline becomes irreversible.

Keywords: caprine, endangered breed, germplasm bank, nonsurgical embryo recovery, NSER

Introduction

The preservation of genetic diversity through breed conservation is an important strategy for preventing species extinction. In the past few centuries, anthropic causes have replaced natural ones as the main reasons for biodiversity loss. The extensive factors involved include unsustainable production and consumption systems, pollution, deforestation, and climate change caused by the emission of greenhouse gases resulting from the burning of fossil fuels.[1] For the past 20 years, ~300 of 6000 farm animal breeds have been declared extinct by the Food and Agricultural Organization. In Brazil, the formation of goat herds began during colonization and specimens have evolved over the years to develop specific adaptability characteristics, resulting in naturalized breeds.[2] However, due to indiscriminate crossbreeding with specialized breeds, genetic variability has been extremely reduced.[2] It is important to emphasize that breed vulnerability can increase as a result of limited geographical distribution, which is the case here. Thus, according to McManus et al.,[3] any naturalized Brazilian goat breeds are at a certain risk, due to both the low number of purebred herds and their limited distribution, often in regions known to have long droughts and harsh conditions. This can result in the definitive loss of alleles that, over time, might be essential for the preservation

[1]Departamento de Patologia e Clínica Veterińria, Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, Brazil.
[2]Molecular Genetics Research Unit, University Center Fametro, Fortaleza, Brazil.
[3]Departmento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife, Brazil.
[4]Faculty of Veterinary, State University of Ceará, Fortaleza, Brazil.
[5]Embrapa Caprinos e Ovinos, Sobral, Brazil.
[i]ORCID ID (https://orcid.org/0000-0002-4872-1718).
[ii]ORCID ID (https://orcid.org/0000-0001-8028-3984).
[iii]ORCID ID (https://orcid.org/0000-0002-4526-0880).

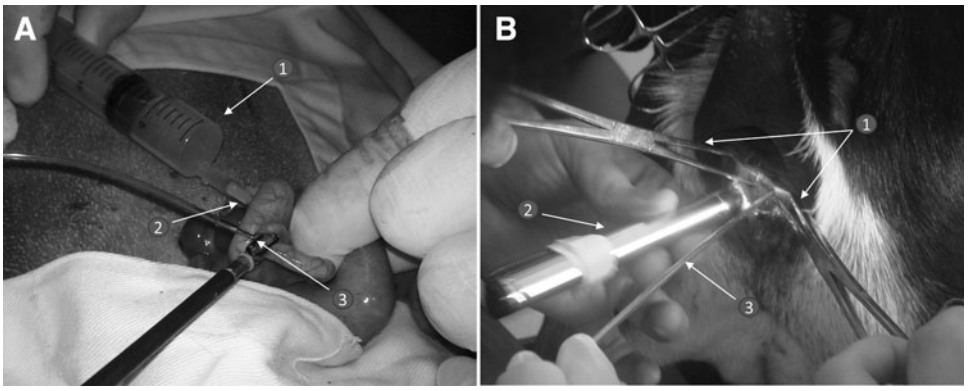

FIG. 1. Embryo recovery in goats by both surgical/laparotomy **(A)** and nonsurgical **(B)** method. **(A)** A syringe with flushing media (1) is inserted in the uterus bifurcation (2) and in the uterotubal junction (3). **(B)** Forceps are inserted in the cervical os (1), which is visualized by a light source (2) while a catheter with a metal mandrel passes through the cervical rings.

of the goat species, especially with the possible impacts of climate change likely to worsen. Among these naturalized breeds, the Canindé breed stands out for being rustic, with high prolificacy and adaptability, allowing for survival and reproduction in a semiarid environment.[4]

Assisted reproductive technologies, such as *in vivo* embryo production followed by cryopreservation, are potential tools for a specimen's conservation.[5] The lower body size of naturalized goats, in comparison with commercial breeds, has resulted in the surgical [laparotomy (LAP)] method becoming the main option for embryo collection. Although LAP can be effective when performed by skilled personnel, it involves anesthesia and surgery risks, and the reproductive tract handling normally results in adherences, impairing future embryo recovery and fertility.[6] As an alternative, therefore, the nonsurgical embryo recovery (NSER) technique has also been successfully reported in goats.[7–9] The simpler anesthetic protocols and reduced nonadhesion formations involved are the main advantages. However, this technique has been mostly used in commercial larger-sized breeds, that is, among which it is theoretically easier to traverse the cervix.[7–9] Despite a comparative study in sheep having favored NSER instead of LAP for embryo recovery,[10] no such comparison is available in goats. Thus, we hypothesized that NSER can be as efficient as LAP for embryo recovery in small-sized Canindé goats. The aim of this study was to compare both embryo collection methods (LAP vs. NSER) in Canindé goats with the aim of creating an embryo bank for later use in a breed conservation program.

Materials and Methods

All protocols have been approved by the Ethics Committee of Animal Use of the University (CEUA/UECE; 1074/2017). The experiment was conducted in the facilities of the Laboratory of Physiology and Control of Reproduction, in Fortaleza (3°43′ S and 38°30′ W), Brazil. Twelve Canindé goats aged 2.8 ± 0.6 years and weighing 31.6 ± 1.4 kg were used as embryo donors. The goats received Tifton (*Cynodon dactylon*) hay, 18% crude protein concentrate, and had *ad libitum* access to water and mineralized salt.

Estrus synchronization was achieved using intravaginal sponges with 60 mg acetate medroxyprogesterone (Progespon, Zoetis, São Paulo, Brazil) for 10 days. On days 8, 9, and 10, five decreasing doses (30/30/20/20/20 mg) of porcine follicle-stimulating hormone (Folltropin V, Vetrepharm, Canada) was administered intramuscularly (im) at 12-hour intervals. On day 9, 50 μg D-cloprostenol (Ciosin, Coopers, Brazil) im was given. Estrus detection and mating began after sponge removal, at 12-hour intervals, using two Canindé males of proven fertility. All female goats in estrus were mated at least twice with both

Table 1. Reproductive Parameters from Canindé Goats Subjected to Superovulation and Embryo Recovery by Either Nonsurgical or Surgical (Laparotomy) Method

Parameter	*NSER*	*LAP*	*Mean/total*	p
Estrous response (%)	100.0 (6/6)	100.0 (6/6)	100.0 (12/12)	—
Does with luteal regression (%)	16.7 (1/6)	33.3 (2/6)	25.0 (3/12)	0.54
Does successfully flushed (%)	100.0 (6/6)	100.0 (6/6)	100.0 (12/12)	—
Procedure duration time (minutes)	32.3 ± 1.4	30.8 ± 1.6	31.6 ± 1.0	0.49
Corpora lutea count per goat (*n*)	13.7 ± 1.6	11.5 ± 1.8	12.6 ± 1.2	0.38
Recovery rate (%)[a]	86.8 ± 5.6	92.8 ± 4.0	89.8 ± 3.4	0.40
Fertilization rate (%)[a]	94.9 ± 3.3	87.0 ± 4.5	90.9 ± 2.9	0.18
Structures recovered per goat (*n*)	11.7 ± 1.3	10.3 ± 1.0	11.0 ± 0.8	0.43
Viable embryos per goat[b] (*n*)	11.2 ± 1.5	8.7 ± 0.7	9.9 ± 0.9	0.15
Freezable embryos per goat[c] (*n*)	10.2 ± 1.1	8.0 ± 0.8	9.1 ± 0.7	0.15
Unfertilized oocytes per goat (*n*)	0.5 ± 0.3	1.7 ± 0.7	1.1 ± 0.4	0.15

() number of animals or structures.
[a]Total average of each goat/rate.
[b]Viable: Grade 1 to 3 (excellent/good, fair, and poor) embryos.
[c]Freezable: Grade 1 and 2 (excellent/good and fair) embryos.
LAP, laparotomy; NSER, nonsurgical embryo recovery.

males. Seven days after the first mating, the ovulatory response was assessed by laparoscopy. Goats were deprived of food and water for 24 hours before embryo collection. Both LAP[10] (Fig. 1A) and NSER[8,9] (Fig. 1B) procedures were similar to those described previously. Recovered embryos were examined under a stereomicroscope (Nikon, Japan) at 40× magnification and evaluated according to the International Embryo Technology Society manual—grades: (1) excellent or good, (2) fair, (3) poor, and (4) dead or degenerating.[11] Embryos were slow-frozen[9] to allow an embryo bank to form for the Canindé breed.

Data are expressed as a percentage (%) or mean ± standard error of mean and analyzed by SPSS v.22 (IBM Corporation, Armonk, NY) software. All results were verified for normality and homoscedasticity using the Shapiro–Wilk and Levene's tests, respectively. When necessary, the data were subjected to Box–Cox transformation. For group comparisons, either Student's *t*-test or Fisher's exact test with $p < 0.05$ considered as significant were drawn upon.

Results

All parameters recorded are listed in Table 1. All goats responded to superovulation and the number of ovulations per female varied from 9 to 20. The number of structures (both recovered and fertilized) along with the embryo quality were not influenced ($p > 0.05$) by the technique. Overall, out of 151 ovulations, 132 structures were collected. The three goats presenting premature luteal regression had similar recovery rates to those showing functional corpora lutea (CL). Regardless of the experimental group, no grade IV embryo (dead or degenerating) was observed.

Discussion

This study compared the LAP and NSER techniques for embryo collection in small-sized Canindé goats, aiming to create an embryo bank for later use in a breed conservation program. In doing so, the study hypothesis was proven, that is, that NSER is as efficient as LAP for embryo recovery in these females. To the best of our knowledge, this is the first study comparing both techniques in goats. There is a general consensus that NSER can contribute to the success of embryo transfer programs, whether for commercial purposes or for the formation of embryo banks to safeguard species/breed preservation. Besides being simple, aligned with animal welfare demands, and presenting a relatively low cost, this technique also allows for avoiding the usual surgical risks associated with LAP.[6]

The high estrous response rate (100%) and ovulatory response (12.6 CL per ewe) indicate that the hormonal treatment was effective in inducing superovulation in Canindé goats.[12] Premature luteal regression occurred in both groups, although this phenomenon is frequently observed in goats[5]—mainly when subjected to superovulation treatment—so is not associated with the technique.

No statistical differences were observed in any parameter related to embryo collection efficiency. The procedure duration time (~32 minutes) is an important parameter if the technique is to be widespread in practice. Moreover, a high recovery rate was obtained in both groups (~90%). This parameter is of utmost importance and resulted in a great number of viable (~9.9) and freezable (~9.1) embryos per goat. Our group has experience in using the NSER in small ruminants.[6,8–10] However, in this study, for the first time we performed the recovery on donor goats weighing <35 kg, resulting in 100% efficiency. The similar efficiency achieved throughout the embryo recovery parameters assessed in our research raises the possibility of abolishing surgical procedures for embryo recovery in goats, even in small-sized breeds. All freezable embryos recovered were slowly frozen as cryopreservation is a valuable tool for animal biobanking. Although embryo production and freezing are more expensive than oocyte/semen preservation, the use of embryos is correlated with faster breed reconstitution.[13]

In conclusion, the NSER method was efficient and can be applied to small-sized Canindé goats. Notably, NSER is simpler than LAP and offers the possibility of successive procedures with few health risks and sequels for females. This study should hopefully boost *in vivo* embryo production programs in the Canindé breed, facilitating the formation of embryo banks, and assuring the availability of genetic diversity before any decline becomes irreversible.

Acknowledgments

The authors thank the LFCR staff for handling the animals. J.M.G.S-F., J.F.F., and V.J.F.F. are CNPq fellows and J.M.G.S-F. is a FAPERJ fellow.

Author Disclosure Statement

No conflicting financial interests exist.

Funding Information

The authors thank for the financial support provided by FUNCAP/PRONEX (Grant No. PR2-0101-00059.01.00/15) and CAPES/COFECUB (Grant No. 88881.142966/2017-01).

References

1. Butchart SH, Walpole M, Collen B, et al. Global biodiversity: Indicators of recent declines. Science 2010;328:1164–1168.
2. Mariante AS, Egito AA. Animal genetic resources in Brazil: Result of five centuries of natural selection. Theriogenology 2002;57:223–235.
3. McManus CM, Hermuche P, Paiva SR, et al. Distribution of goat breeds in Brazil and their relationship with environmental controls. Biosci J 2014;30:1819–1836.
4. Maia MS, Costa AN. Evaluation of reproductive performance of a herd of Canindé goats in the semi-arid zone of Rio Grande do Norte, Brazil. Rev Bras Zootec 1996;26:46–53.
5. Souza-Fabjan JMG, Pereira AF, Melo CHS, et al. Assessment of the reproductive parameters, laparoscopic oocyte recovery and the first embryos produced in vitro from endangered Canindé goats (*Capra hircus*). Reprod Biol 2013;13:325–332.
6. Fonseca JF, Oliveira MEF, Brandão FZ, et al. Nonsurgical embryo transfer in goats and sheep: The Brazilian experience. Reprod Fertil Dev 2019;31:17–26.
7. Pereira RJTA, Sohnrey B, Holtz W. Nonsurgical embryo collection in goats treated with prostaglandin F2-alpha and oxytocin. J Anim Sci 1998;76:360–363.

8. Fonseca JF, Zambrini FN, Alvim GP, et al. Embryo production and recovery in goats by non-surgical transcervical technique. Small Rumin Res 2013;111:96–99.
9. Fonseca JF, Batista RITP, Souza-Fabjan JMG, et al. Freezing goat embryos at different developmental stages and quality using ethylene glycol and a slow cooling rate. Arq Bras Med Vet Zootec 2018;70:1489–1496.
10. Santos JDR, Ungerfeld R, Balaro MFA, et al. Transcervical vs. Laparotomy embryo collection in ewes: The effectiveness and welfare implications of each technique. Theriogenology 2020;153:112–121.
11. Stringfellow DA, Givens MD. *Manual of the International Embryo Transfer Society*. IETS, Ed 4, Champaign, IL; 2010.
12. Moura RR, Lopes-Junior ES, Teixeira DIA, et al. Pronuclear embryo yield in Canindé and Saanen goats for DNA microinjection. Reprod Dom Anim 2010;45:e101–e106.
13. Boettcher PJ, Stella A, Pizzi F, et al. The combined use of embryos and semen for cryogenic conservation of mammalian livestock genetic resources. Genet Sel Evol 2005; 37:657–675.

Address correspondence to:
Joanna M.G. Souza-Fabjan, JMG
Departamento de Patologia e Clínica Veterinária
Faculdade de Veterinária
Universidade Federal Fluminense
Rua Vital Brazil Filho, 64
Niterói, RJ CEP 24230-340
Brazil

E-mail: joannavet@gmail.com