Received: 18 June 2019 | Accepted: 7 August 2019

DOI: 10.1111/rda.13550

SHORT COMMUNICATION

Reproduction in Domestic Animals WILEY

Pregnancy rate after fixed-time transfer of cryopreserved embryos collected by non-surgical route in Lacaune sheep

Lucas Machado Figueira[1,2] | Nadja Gomes Alves[1] | Ribrio Ivan Tavares Pereira Batista[2] | Viviane Lopes Brair[2] | Renato Ribeiro Lima[1] | Maria Emilia Franco Oliveira[3] | Jeferson Ferreira Fonseca[4] | Joanna Maria Gonçalves Souza-Fabjan[2]

[1]Universidade Federal de Lavras, Minas Gerais, Brazil

[2]Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Niterói, Brazil

[3]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Jaboticabal, Brazil

[4]Embrapa Caprinos e Ovinos, Núcleo Regional Sudeste, Coronel Pacheco, Brazil

Correspondence
Joanna Maria Gonçalves Souza-Fabjan, Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Niterói, Brazil.
Email: joannavet@gmail.com

Funding information
FAPEMIG, Grant/Award Number: Project CVZ-PPM 00201-17; FAPERJ, Grant/Award Number: Project E-26/203.168/2017; Embrapa, Grant/Award Number: Project 02.08.02.005.00.05

Abstract

This study investigated the feasibility of applying fixed-time (cryopreserved) embryo transfer in ewes. Embryos (n = 106) were non-surgically recovered from superovulated donors (n = 39) on day 6–7 after oestrus. Straws containing one or two embryos (morulae and/or blastocysts) subjected to either slow freezing (SF, n = 62) or vitrification (VT, n = 44) were randomly used within fixed-time embryo transfer on Day 8.5. Recipient ewes were nulliparous (n = 58) bearing corpora lutea after synchronous oestrous induction protocol. The pregnancy rate was higher (p = .03) in SF (39.4%) than VT (16.9%) and survival rate tended (p = .08) to be higher in SF than in VT (25.8% vs. 15.9%). Lambing rates were similar (p = .13) between SF (20.9%) and VT (15.9%). Embryos recovered by non-surgical route after cervical dilation treatment and later cryopreserved by either slow freezing or vitrification produced reasonable pregnancy rates after FTET.

KEYWORDS
embryo cryopreservation, multiple ovulation and embryo transfer, ovine

1 | INTRODUCTION

Non-surgical embryo recovery (NSER) by cervical route has been proposed to overcome the limitation of successive laparotomy procedures beyond animal welfare concerns. However, hormonal treatments for cervical dilation are necessary to produce efficient NSER. Our team's use of d-cloprostenol, estradiol benzoate and oxytocin has led to the achievement of promising cervical transposition and uterine flushing results in Brazilian native and Lacaune breeds (Fonseca et al., 2019). Previous studies demonstrated that some of the hormones used for cervical dilation (PGE2 and estradiol) did not inhibit embryo development or metabolism (McKelvey et al., 1997) and had no effect on foetal viability and growth after the fresh embryos were transferred (Mylne, Dingwall, King, McKelvey, & Robinson, 1999). However, the viability of these embryos following different cryopreservation techniques has yet to be evaluated. Thus, this study aimed to assess the survival capacity of non-surgically recovered and cryopreserved (vitrification or slow freezing) ovine embryos after fixed-time transfer.

2 | MATERIALS AND METHODS

2.1 | Experimental conditions

This research was approved by the Animal Care Committee of Embrapa Dairy Cattle (#2512100516/2016). The experiment was performed during the breeding season on a commercial farm in Soledade de Minas (latitude 22°3′ S). Animals were kept in collective pens and received a diet of two meals with mineral salt and water ad libitum.

2.2 | Non-surgical embryo recovery and cryopreservation

Lacaune donors (n = 39; 68.3 ± 6.7 kg of body weight, 3.5 ± 0.2 of body condition score and 130.4 ± 5.4 days in milk) were superovulated and mated. Embryos were recovered by transcervical method (Fonseca et al., 2019) 6–7 days after oestrus. Cervical dilation treatment was based on 1 mg estradiol benzoate (Sincrodiol®, OuroFino) and 37.5 μg d-cloprostenol (Prolise®, Tecnopec) i.m. at 16 hr and 50 IU oxytocin (Ocitocina forte®, UCB, Brazil) i.v. 20 min before NSER. Embryo morphology was classified according to the IETS. Grade I and II embryos randomly underwent either slow freezing (SF; Fonseca et al., 2018) or vitrification (VT; Gibbons, Bruno-Galarraga, Fernandez, Gonzalez-Bulnes, & Cueto, 2019). The percentage of morulae and blastocysts transferred did not differ (p < .05, chi-square test) in SF (48.4%–30/62 and 51.6%–32/62) and VT (36.4%–16/44 and 63.6–28/44) groups, respectively.

2.3 | Preparation and evaluation of recipients

Recipients received intravaginal sponges for 6 days (60 mg medroxyprogesterone acetate, Progespon®, Zoetis), and 200 IU eCG (Folligon 5000 IU®, Intervet) i.m. and 37.5 μg d-cloprostenol (Sincrocio®, OuroFino) i.m. was administered 24 hr before sponge removal (Day 0). Transrectal ultrasonography (M5VET®, Mindray– 8.0 MHz) was performed 24 hr before embryo transfer to detect the number and size of the corpora lutea (CL).

2.4 | Fixed-time embryo transfer

Embryo transfer was performed using the semi-laparoscopic technique on Day 8.5 to the horn *ipsilateral* to the CL. After 24 hr fasting, recipients that presented at least 1 CI (92%; 58/63) received one or two cryopreserved embryos (morulae and/or blastocysts) (Fonseca et al., 2018). Pregnancy rate (number of pregnant recipients/number of recipients receiving embryos) and embryo survival rate (number of embryos with heartbeat/number of embryos transferred) were recorded by ultrasonography on Day 31. Lambing rates (number of lambs born/number of embryos transferred) were also assessed.

2.5 | Statistical analyses

Data of pregnancy, survival and lambing rates were analysed using SAS® program. The PROC GLIMMIX with binomial distribution and logit link function was used. The model for pregnancy rate included cryopreservation technique, type of transferred straw (1: one morula, 2: one blastocyst, 3: two morulae 4: two blastocyst and 5: morula and blastocyst) and number of CL as fixed effects. Values of p < .05 were considered significant and p < .10 a tendency. Data are presented as percentage and mean ± *SEM*.

3 | RESULTS

The number of CL per recipient was 1.3 ± 0.1 in SF and 1.4 ± 0.1 in VT, and the number of embryos transferred was 1.9 ± 0.1 in SF and 1.8 ± 0.1 in VT. Regardless of the cryopreservation technique employed, the embryos that were recovered by non-surgical method after cervical dilation treatment established and sustained pregnancy (Table 1). Lambing rates were similar (p> .05) between treatments; however, transfer of embryos from the VT treatment had lower (p < .05) pregnancy and tended (p = .08) to have lower survival rate.

4 | DISCUSSION

For the first time, we demonstrated that ovine embryos recovered by NSER (after exogenous hormonal cocktail) and subjected to cryopreservation can establish reasonable rates of pregnancy. The overall survival efficiency after cryopreservation was 21.7%, and VT group had lower pregnancy rate and tended to have lower survival rate than SF group. The survival rate of in vivo-derived cryopreserved embryos by vitrification technique described in the existing literature is 32.0%–36.0% (Folch, Olivera Muzante, & Aguilar Gomez, 2000), 41.2%–50.0% (Gibbons, Cueto, & Pereyra-Bonnet, 2011) or 60.1%–75.1% (Dattena et al., 2004). In the present study, the VT embryo survival rate was 15.9%, which is similar to the 22.6% rate that was recently reported for embryos recovered by laparotomy (Gibbons et al., 2019). However, our lambing rates (18.8%) were lower than the 62.9% rate observed by Dattena, Ptak, Loi, and Cappai (2000) and the 80.0% rate recorded by Naitana et al., (1995). One potential

TABLE 1 Pregnancy, survival and lambing rates after transfer of embryos recovered by transcervical route and cryopreserved by either slow freezing or vitrification

Cryopreservation technique	Pregnancy (%)	Survival (%)	Lambing (%)
Slow Freezing	39.4 (13/33)	25.8 (16/62)	20.9 (13/62)
Vitrification	16.0 (4/25)	15.9 (7/44)	15.9 (7/44)
Average/total	29.3 (17/58)	21.7 (23/106)	18.8 (20/106)
p-value	.03	.08	.13

Note: Fixed-time embryo transfer was performed on Day 8.5 after sponge removal; Pregnancy: number of pregnant ewes/number of recipient ewes receiving embryos; Survival: number of embryos with heartbeat/number of transferred embryos; Lambing: number of lambs born/number of transferred embryos.

explanation for this discrepancy is that these authors only transferred blastocysts that re-expanded after vitrification/rewarming.

Similar pregnancy rates were obtained in the current study on SF embryos (39.4%) as those achieved in a previous study in which embryos were surgically recovered (34.8%; Green, Santos, Sicherle, Landim-Alvarenga, & Bicudo, 2009). Establishment of pregnancy in ruminants depends on optimal interaction between the developing embryo-conceptus and the maternal uterine environment (Randi et al., 2016). Early-stage embryo-transfer studies in sheep (Naitana et al., 1995) and cattle (Rowson, Lawson, Moor, & Baker, 1972) have demonstrated the need for a close synchrony (±24 hr) between the donor and recipient, relative to oestrous onset. Pregnancy rates following the transfer of Day 8 bovine embryos to asynchronous (Day 5) or synchronous (Day 8) recipients were 4.8% and 61.1%, respectively, highlighting the low tolerance for an out-of-sync uterine environment (Geisert et al., 1991). The current study employed the FTET technique; this frequently does not take into account oestrous behaviour and ovulation, resulting in lower pregnancy rates in comparison with when oestrous behaviour and ovulation are assessed. This negative effect of asynchrony between recipient and embryo transfer appears to predominantly impact the VT embryos.

In conclusion, embryos recovered by non-surgical method after cervical dilation treatment and later cryopreserved are able to sustain a pregnancy after FTET, regardless of the cryopreservation technique used.

ACKNOWLEDGEMENTS

The authors thank Embrapa (Project 02.08.02.005.00.05), FAPEMIG (Project CVZ-PPM 00201-17) and FAPERJ for financial support. JMGS-F is a FAPERJ fellow, and MEFO and JFF are CNPq fellows.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

LMF developed the experimental activities, analysed the data and wrote the manuscript; RITPB, VLB, JMGS-F and JFF developed the experimental activities; RRL, NGA, MEFO and JMGS-F analysed the data and corrected the manuscript; JFF and JMGS-F designed and coordinated the study.

DATA AVAILABILITY

The data on which the findings of this study were drawn are available from the corresponding author upon request.

ORCID

Renato Ribeiro Lima https://orcid.org/0000-0003-4607-4964

Maria Emilia Franco Oliveira https://orcid.org/0000-0002-7730-290X

Jeferson Ferreira Fonseca https://orcid.org/0000-0001-8028-3984

Joanna Maria Gonçalves Souza-Fabjan https://orcid.org/0000-0002-4872-1718

REFERENCES

Dattena, M., Accardo, C., Pilichi, S., Isachenko, V., Mara, L., Chessa, B., & Cappai, P. (2004). Comparison of different vitrification protocols on viability after transfer of ovine blastocysts in vitro produced and in vivo derived. *Theriogenology*, *62*, 481–493. https://doi.org/10.1016/j.theriogenology.2003.10.010

Dattena, M., Ptak, G., Loi, P., & Cappai, P. (2000). Survival and viability of vitrified in vitro and in vivo produced ovine blastocysts. *Theriogenology*, *53*, 1511–1519.

Folch, J., Olivera Muzante, J., & Aguilar Gomez, B. (2000). *Resultados obtenidos em la transferência de embriones dentro del programa genético de La U.P.R.A. carnes Oviaragon*. In: Resumenes de las XXV Jornadas SEOC; 559–561.

Fonseca, J. F., Batista, R. I. T. P., Souza-Fabjan, J. M. G., Oliveira, M. E. F., Brandão, F. Z., & Viana, J. H. M. (2018). Freezing goat embryos at different developmental stages and quality using ethylene glycol and a slow cooling rate. *Arquivo Brasileiro De Medicina Veterinária E Zootecnia*, *70*, 1489–1496. https://doi.org/10.1590/1678-4162-10196

Fonseca, J. F., Oliveira, M. E. F., Brandão, F. Z., Batista, R. I. T. P., Garcia, A. R., Bartlewski, P. M., & Souza-Fabjan, J. M. G. (2019). Non-surgical embryo transfer in goats and sheep: The Brazilian experience. *Reproduction, Fertility and Development*, *31*, 17–26. https://doi.org/10.1071/RD18324

Geisert, R. D., Fox, T. C., Morgan, G. L., Wells, M. E., Wettemann, R. P., & Zavy, M. T. (1991). Survival of bovine embryos transferred to progesterone-treated asynchronous recipients. *Journal of Reproduction and Fertility*, *92*, 475–482. https://doi.org/10.1530/JRF.0.0920475

Gibbons, A., Bruno-Galarraga, M., Fernandez, J., Gonzalez-Bulnes, A., & Cueto, M. (2019). Vitrified embryo transfer in merino sheep under extensive conditions. *Animal Reproduction*, *16*, 297–301. https://doi.org/10.21451/1984-3143-AR2018-0108

Gibbons, A., Cueto, M., & Pereyra-Bonnet, F. (2011). A simple vitrification technique for sheep and goat embryo cryopreservation. *Small Ruminant Research*, *95*, 61–64. https://doi.org/10.1016/j.smallrumres.2010.08.007

Green, R. E., Santos, B. F. S., Sicherle, C. C., Landim-Alvarenga, F. C., & Bicudo, S. D. (2009). Viability of OPS vitrified sheep embryos after direct transfer. *Reproduction in Domestic Animals*, *44*, 406–410. https://doi.org/10.1111/j.1439-0531.2008.01088.x

McKelvey, W. A. C., McEvoy, T. G., Dingwall, W. S., Robinson, J. J., Lindsay, E., King, M. E., … Mylne, M. J. A. (1997). The use of exogenous hormones to facilitate transcervical embryo recovery in sheep and their effect on embryo development. *Theriogenology*, *47*, 369. https://doi.org/10.1016/S0093-691X(97)82496-X

Mylne, M. J. A., Dingwall, W. S., King, M. E., McKelvey, W. A. C., & Robinson, J. J. (1999). The viability of ovine embryos recovered transcervically and transferred by laparoscope. *Proceedings of the British Society of Animal Science*, *1999*, 16–16. https://doi.org/10.1017/S175275620000171X

Naitana, S., Dattena, M., Gallus, M., Loi, P., Branca, A., Ledda, S., & Cappai, P. (1995). Recipient synchronization affects viability of

vitrified ovine blastocysts. *Theriogenology*, *43*, 1371–1378. https://doi.org/10.1016/0093-691X(95)00122-O

Randi, F., Fernandez-Fuertes, B., McDonald, M., Forde, N., Kelly, A. K., Bastos Amorin, H., … Lonergan, P. (2016). Asynchronous embryo transfer as a tool to understand embryo-uterine interaction in cattle: Is a large conceptus a good thing? *Reproduction, Fertility and Development*, *12*, 1999–2006. https://doi.org/10.1071/RD15195

Rowson, L. E., Lawson, R. A., Moor, R. M., & Baker, A. A. (1972). Egg transfer in the cow: Synchronisation requirements. *Journal of Reproduction and Fertility*, *28*, 427–431. https://doi.org/10.1530/JRF.0.0280427

How to cite this article: Figueira LM, Alves NG, Batista RITP, et al. Pregnancy rate after fixed-time transfer of cryopreserved embryos collected by non-surgical route in Lacaune sheep. *Reprod Dom Anim*. 2019;54:1493–1496. https://doi.org/10.1111/rda.13550