The 3rd CZU Prague hybrid seminar
"Biotechnology in small ruminant reproduction: an international experience"

Non-surgical artificial insemination, embryo recovery and transfer in small ruminants

Joanna Mª G. Souza-Fabjan

Universidade Federal Fluminense

Niterói, Rio de Janeiro, Brazil

Online, Friday, May 3, 2024; 13:30-14:00

2014-present: Fluminense Federal University

Niterói, in Rio de Janeiro State

You are all invited to visit us!

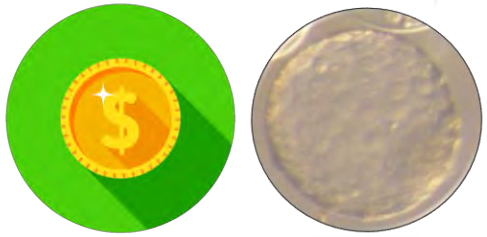

LIBERTADORES
2024

AI and MOET – tools for genetic improvement:

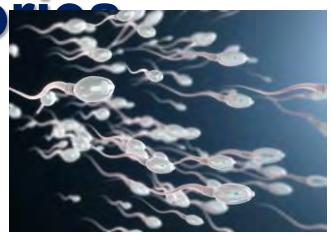
- Pivotal for the propagation of animals with high genetic and economic merit
- Facilitate international trade
- Contribute to salvaging endangered animal populations

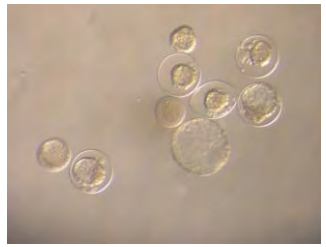
AI

MOET



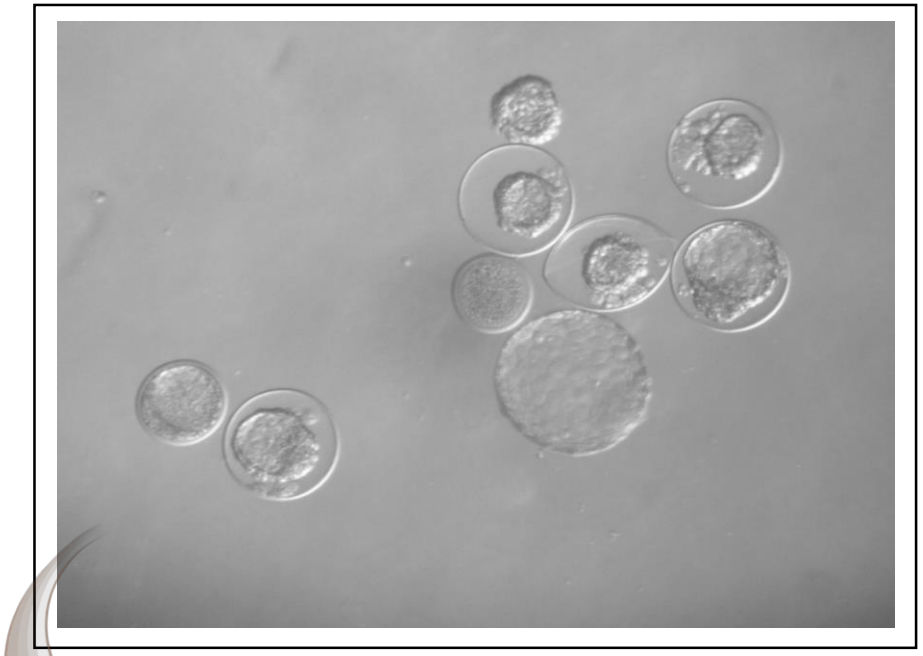
Germplasm biorepositories





Embryo production

***In vivo* embryo production / MOET / ET**



BIOPRESERVATION AND BIOBANKING
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DOI: 10.1089/bio.2020.0125

NonSurgical Embryo Recovery from Estrus-Synchronized or Superovulated Morada Nova Ewes: A Feasible Strategy for Sheep Embryo Banking

Aline Matos Arrais,[1] Marco Roberto Bourg de Mello,[1] Gabriel Brun Vergani,[2] Lucas Machado Figueira,[3] Sérgio Novita Esteves,[4] Verônica Schinaider do Amaral Pereira,[4] Pawel Mieczyslaw Bartlewski,[5] Maria Emilia Franco Oliveira,[2] Joanna Maria Gonçalves Souza-Fabjan,[3] and Jeferson Ferreira da Fonseca[6]

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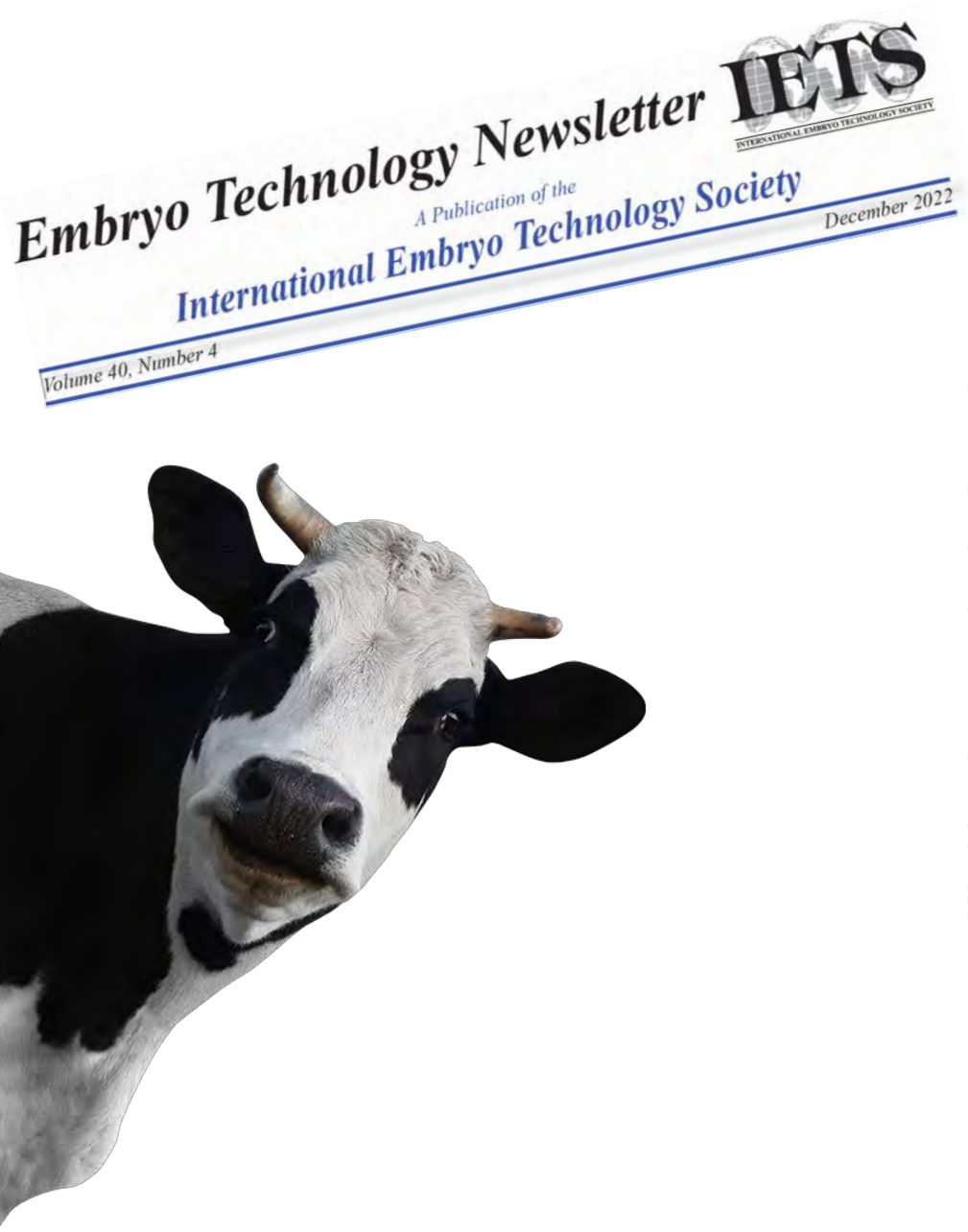
Nonsurgical Embryo Recovery as a Feasible Tool for Supporting Embryo Biobanks of Locally Adapted Brazilian Sheep and Goats

Jeferson F. Fonseca,[1,i] Gabriel B. Vergani,[2,ii] Monalisa S.D. Lima,[3] Kleibe M. Silva,[1] Alexandre W.U. Monteiro,[1] Alexandre F. Ramos,[4,iii] Bruna R.C. Alves,[5,iv] Joanna M.G. Souza-Fabjan,[5,v] Maria E.F. Oliveira,[1,2] and Ribrio I.T.P. Batista[5]

Higher cryotolerance: **preferred** for establishing biobanks



MOET vs IVP: Bovine world embryo production 2002-2021

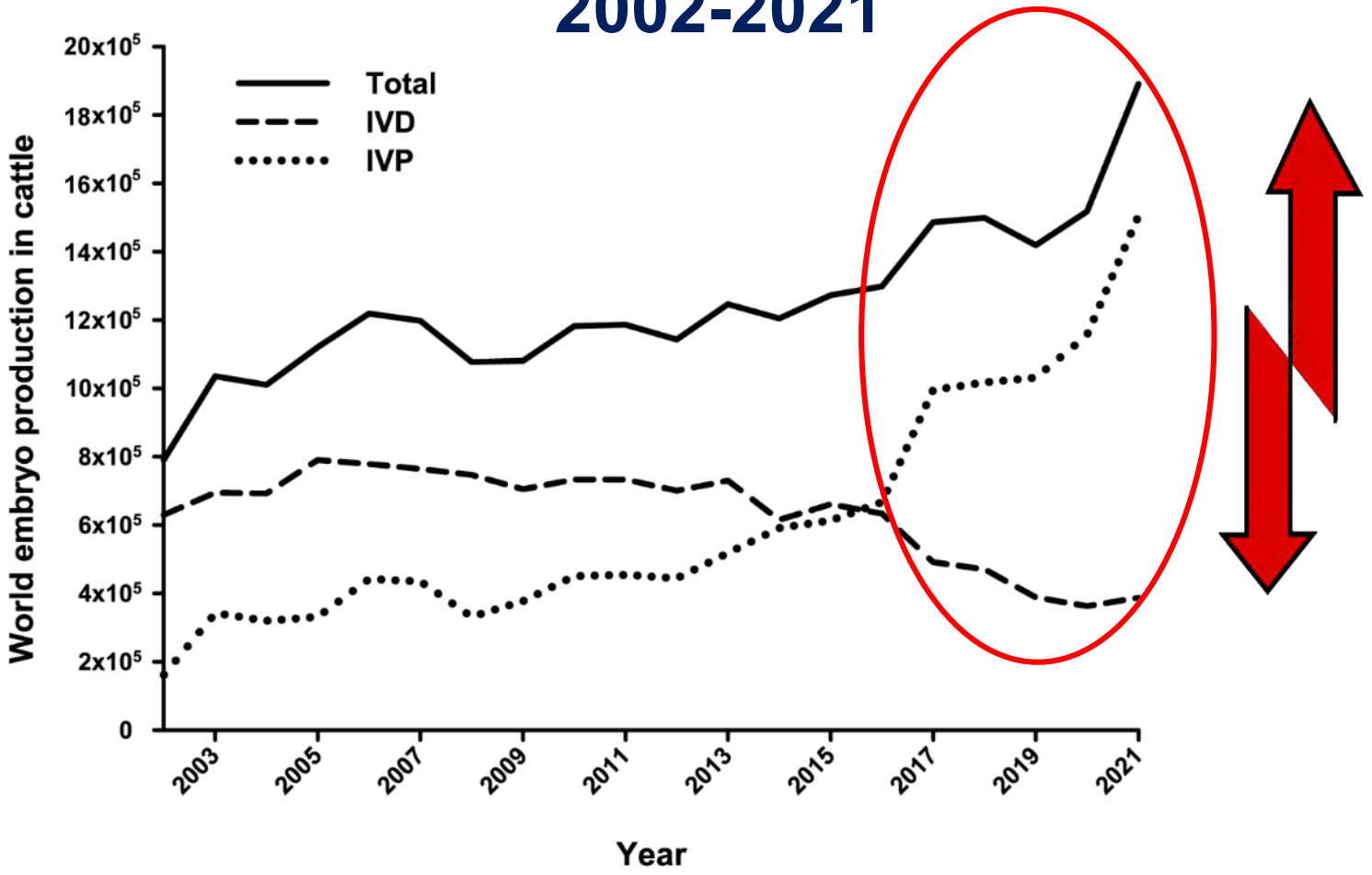


Figure 2. Number of bovine embryos [*in vivo* derived (IVD), *in vitro* produced (IVP), and total] recorded in the period 2002–2021

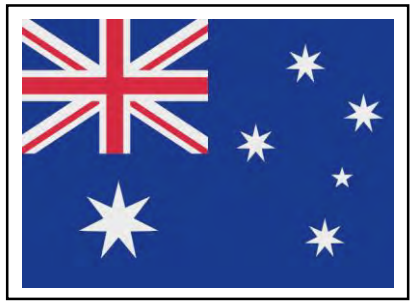
Sheep embryo production in 2021

Table 10. Sheep: *in vivo* derived [IVD] and *in vitro* produced [IVP] embryo collections and transfers in 2021

Region/ Country	IVD Embryos					IVP embryos					
	Flushes	Embryos	Embryo transfer			Donors	Oocytes	Embryos	Embryo transfer		
			Fresh	Frozen					Fresh	Frozen	
				Domestic	Foreign					Domestic	Foreign
Europe											
Greece	3	13	0	0	0	0	0	0	0	0	0
Romania	2	27	27	0	0	0	0	0	0	0	0
Russian Fed.	0	0	0	0	1,878	0	0	0	0	0	0
Serbia	4	32	0	6	0	0	0	0	0	0	0
Spain	33	320	0	60	0	18	384	180	0	0	0
UK	167	993	27,593	641	0	0	0	0	0	0	0
Total	209	1,385	27,620	707	1,878	18	384	180	0	0	0
N America											
Canada	24	76	28	414	6	0	0	0	0	0	0
Mexico	83	589	224	125	0	0	0	0	0	0	0
USA	1,623	9,619	7,462	768	0	156	1,620	446	353	93	0
Total	1,730	10,284	7,714	1,307	6	156	1,620	446	353	93	0
Oceania											
Australia	2,924	21,878	19,536	859	0	0	0	0	0	0	0
Total	2,924	21,878	19,536	859	0	0	0	0	0	0	0
S America											
Brazil	918	7636	5902	1311	0	0	0	0	0	0	0
Total	918	7,636	5,902	1,311	0	0	0	0	0	0	0
Grand Total	**5,781**	**41,183**	**60,772**	**4,184**	**1,884**	**174**	**2,004**	**626**	**353**	**93**	**0**

98.5% are MOET

MOET



~7.1 embryos/ewe

Embryo Technology Newsletter
A Publication of the
International Embryo Technology Society
Volume 40, Number 4
December 2022

Goat embryo production in 2021

Table 11. Goats: *in vivo* derived [IVD] and *in vitro* produced [IVP] embryo collections and transfers in 2021

Region/ Country	IVD Embryos					IVP embryos					
	Flushes	Embryos	Embryo transfer			Donors	Oocytes	Embryos	Embryo transfer		
			Fresh	Frozen					Fresh	Frozen	
				Domestic	Foreign					Domestic	Foreign
Europe											
France	0	0	0	3	0	0	0	0	0	0	0
Spain	48	648	0	200	0	25	504	268	0	0	0
Total	48	648	0	203	0	25	504	268	0	0	0
N America											
Canada	15	43	4	0	0	0	0	0	0	0	0
Mexico	0	0	0	0	0	25	312	87	87	0	0
USA	1,199	8,646	9,131	417	0	942	22,175	6,000	4,026	1,489	0
Total	1,214	8,689	9,135	417	0	967	22,487	6,087	4,113	1,489	0
Oceania											
Australia	189	1,856	540	0	0	0	0	0	0	0	0
Total	189	1,856	540	0	0	0	0	0	0	0	0
Grand Total	**1,451**	**11,193**	**9,675**	**620**	**0**	**992**	**22,991**	**6,355**	**4,113**	**1,489**	**0**

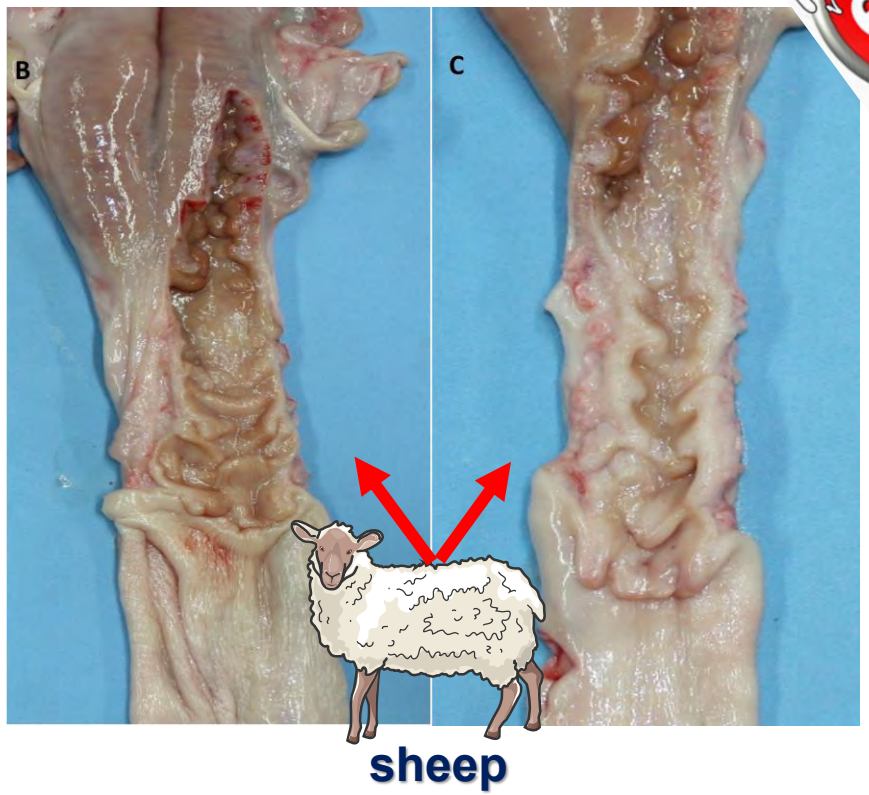
MOE
T

~7.7 embryos/doe

64% are MOET

Small ruminant particularities

- Small body size – limits the rectal palpation to **digital exams**
- Uterine cervix – difficult to penetrate with most catheters



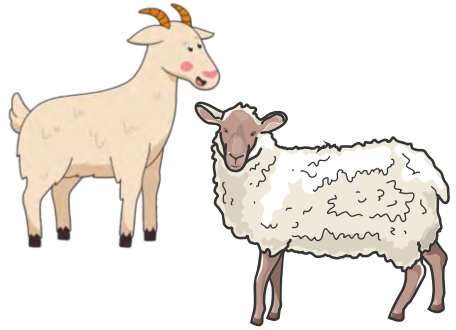
sheep

Ewes have long (4–8 cervical rings), rigid, and tortuous cervix

Fig. 1. The classification of the appearance of the external os of the ewe (a) duckbill, (b) slit, (c) rose, (d) papilla, and (e) flap.

Kershaw et al., 2005

AI techniques in small ruminants



Laparotomy

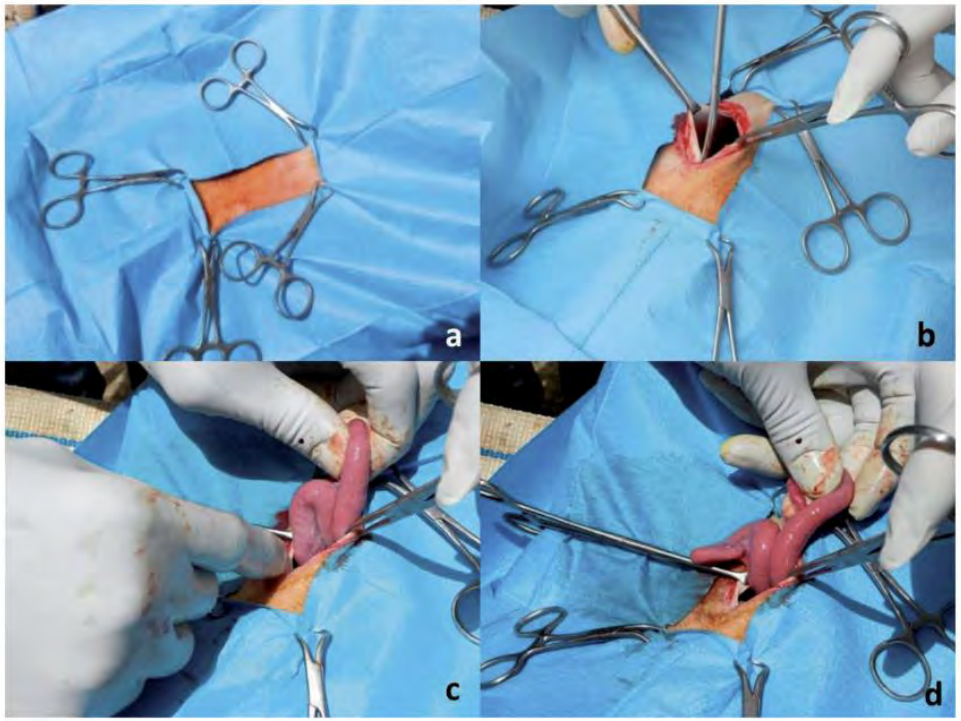


Figure 1. Preparation of the patient for artificial insemination. (a) Surgical scrub and delimitation of the incision area, cranially to the udder; (b) incision of 5 cm length of the abdomen wall, along the white line; (c) exposure of the uterine horns; (d) digital palpation of uterine horns for assessing the tone and content.

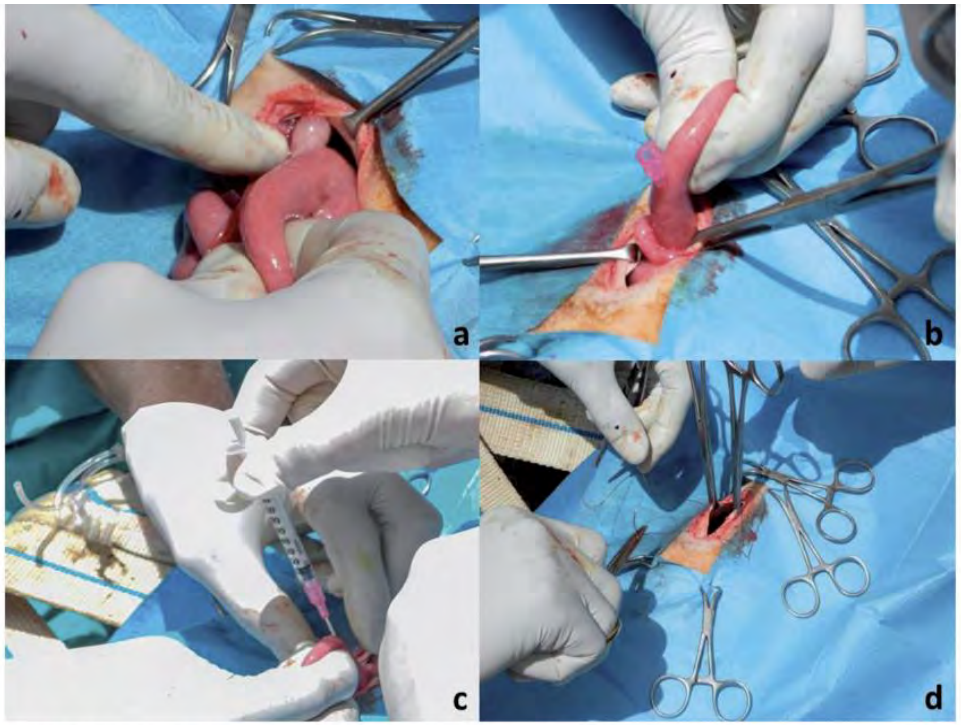
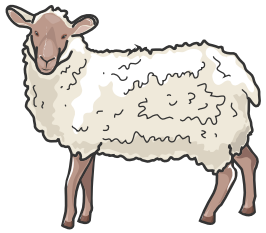


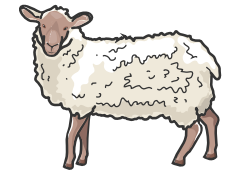
Figure 2. Artificial intra-uterine insemination. (a) exposure of ovaries and check for pre-ovulatory follicles; (b) insertion of a 18-gauge intravenous catheter into the uterine wall; (c) deposition of thawed semen into the uterine lumen; (d) abdomen wall closure.

Lopyrin and Loginova, 1958;
Salamon and Lightfoot, 1967;
Silla et al., 2021



AI techniques in sheep

most widespread



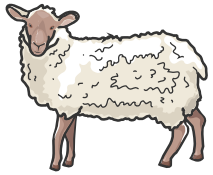
Laparoscopy

Killen and Caffery, 1982;
Maxwell et al., 1984;
Rocha et al., 2022

Pericervical/vaginal

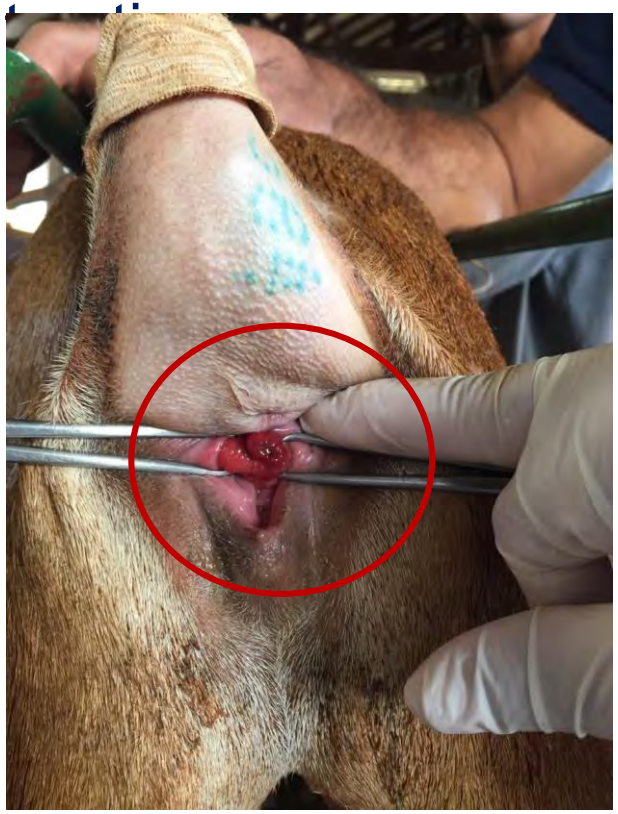
Photo provided by Alejo Menchaca

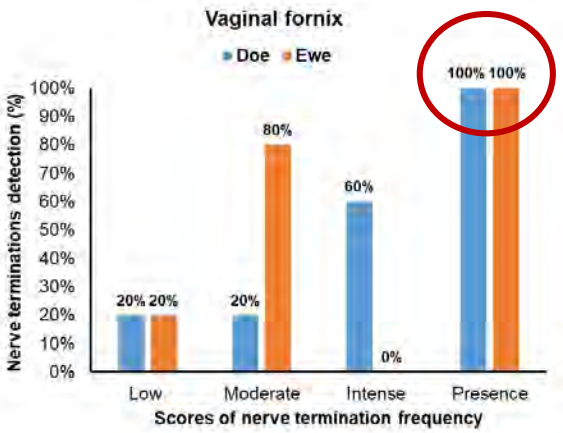
Lopyrin and Loginova, 1958;
Salamon and Lightfoot, 1967;
Menchaca et al., 2005

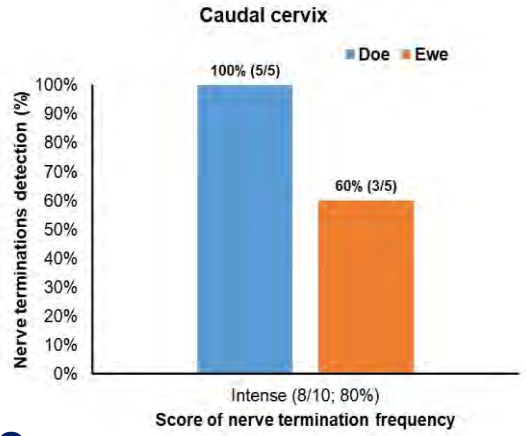


AI techniques in sheep

Transcervical, usually with cervical







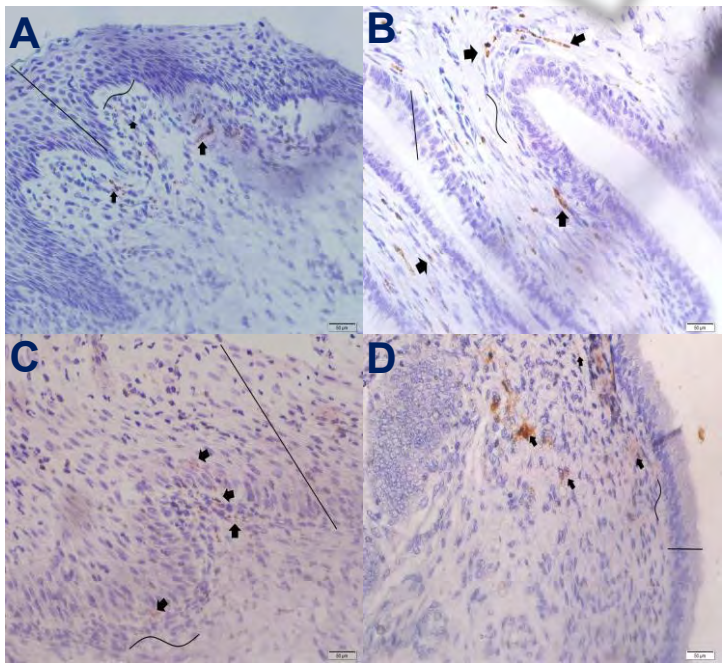


Fig. Histological sections of the vaginal fornix and cervix submitted to immunohistochemical staining by reaction with PGP-9.5 antibody. Black arrow = nerve terminations; Straight line = epithelium; Wavy line = loss of connective tissue.

Nerve terminations?

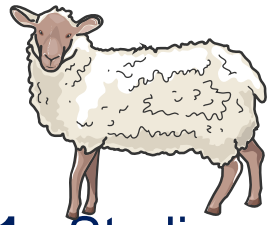


Table 1. Studies conducted in sheep aimed at increasing the penetrability of the uterine cervix during transcervical AI procedures.

Guelph system

(Candappa & Bartlewski, 2011)

Breed/Parity	Strategy/Hormonal protocol	Efficiency (%) Intrauterine penetration Treatment*	Intrauterine penetration Control*	Pregnancy Treatment*	Pregnancy Control*	Overall outcomes	Reference
Suffolk, Dorset, Cheviot, Suffolk crossbred, Leicester, Clun Forest, and Hampshire cross/multiparous	Guelph system of AI (different specula, forceps, instruments; dorsal recumbency restraint + cervical traction)	82 (73/89)	ND	ND	ND	High uterine penetration by using cervical traction	Halbert et al., 1990a
Suffolk and white-faced/ diverse	Guelph system of AI	54 (49/90)	ND	80 (72/90)[A,B]	ND	Higher pregnancy when AI was intrauterine, compared to mid cervical (88 x 57%)	Halbert et al., 1990b
Rambouillet and crossbreds/ multiparous	Flexible catheter (designed to allow semen deposition in the uterine horn) and retroload AI gun	ND	ND	5 (5/99)[B]	ND	When depositing small numbers of sperm, it reduced pregnancy compared to LAI	Wulster-Radcliffe et al., 2004
Rasa Aragonesa/ multiparous	Antiretrograde flow device for sheep cervical AI (DARIO)	ND	ND	59 (390/662)[B]	50 (316/637)[B]	DARIO avoided visual cervix injuries, decreased retrograde flow de visu, and increased fertility rate	Macias et al., 2017
Sarda/ multiparous	Surgical incision of cervical folds	90 (35/39)	ND	72 (28/39)[A]	ND	Facilitated the transcervical passage and intrauterine semen deposition, resulting in pregnancy rate similar to LAI (72 vs 70%)	Pau et al., 2020
Dorset, Rambouillet, Hampshire, and Suffolk/diverse	200-600 USP OT (i.v.)	77 (33/43)	0 (0/15)	ND	ND	OT allowed a greater cervical penetration, but fertility was not assessed	Khalifa et al., 1992
Crossbred/ multiparous	200 USP OT (i.v.)	ND	ND	51 (28/55)[A] with LAI	66 (36/55)[A] with LAI	OT and cervical manipulation both decreased fertilization rate (47 vs 59%) and the former affected fertility after LAI	Stellflug et al., 2001
Welsh Mountain, Île-de-France, Vendéenne, Romanov and Sarda/multiparous	2 mg oFSH (i.c.) 2 mg misoprostol, PGE1 (i.c.) 2 mg oFSH + 300 IU OT (i.c.) Ram effect	ND ND	ND ND	ND	ND	Cervical relaxation was enhanced by the presence of a ram but not by any drug used	Falchi et al., 2012
Rideau Arcott x Polled Dorset/ multiparous	Guelph system of AI + controlled slow-release vaginal inserts of PGE2 (Cervidil®)	90 (36/40)	75 (30/40)	3 (1/40)[A]	8 (3/40)[A]	Reduced the time to penetrate the cervix in the reproductive season, but reached similar pregnancy rates	Bartlewski and Candappa, 2015
Welsh Mountain/ multiparous	2 mg oFSH (i.c.) 1 mg misoprostol, PGE1 (i.c.)	100 (9/9) 100 (9/9)	ND	ND	ND	Either oFSH or misoprostol facilitated cervical penetration, but their combination had no benefit	Leethongdee et al., 2007
Rideau Arcott, Rideau Arcott x Suffolk/multiparous	5 µg human interleukin-α 8 (vaginal suppository)	40 (2/5)	0 (0/2)	ND	ND	Not sufficient to relax the cervix	Croy et al., 1999
Kivircik/ diverse	0.5 mg carazolol (i.m.)	0 (0/150)	0 (0/150)	63 (95/150)[A,B]	57 (85/150)[A,B]	Increased the rate of ewes in which deep penetration of cervix was achieved (48 vs 33%), but did not affect lambing rate	Gündüz et al., 2010

Abbreviations: i.v. = intravenous; i.c. = intra-cervical; i.m. = intramuscular; N/D = not determined; AI = artificial insemination; LAI = laparoscopic AI; OT = oxytocin; oFSH = ovine follicle-stimulating hormone; PGE = prostaglandin E; USP = units of oxytocin.

***Treatment: strategy/hormones applied to enhance cervical penetrability; Control: respective controls for the tretament applied; ( ) number of animals;**

[A]: frozen-thawed semen.

[B]: chilled semen.

Pregnancy rates after AI

- Pregnancy rates are generally lower when F/T rather than fresh ram semen is deposited in the cervical os (~ 30 vs. 75%)
  - Exact cause of impaired F/T sperm cervical transit?

(Salamon and Maxwell, 2000)

- Cryopreservation-induced alterations in seminal plasma molecules (proteins, lipids, and RNAs)

(Reviewed by Fair et al., 2019; Warr et al., 2022)

Abril-Parreño et al., 2021

REPRODUCTION
REVIEW

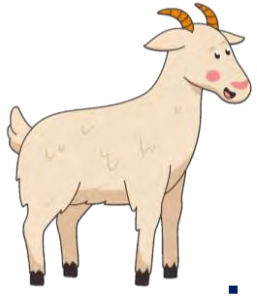
The biological mechanisms regulating sperm selection by the ovine cervix

S Fair[1], K G Meade[2], K Reynaud[3], X Druart[3] and S P de Graaf[4]

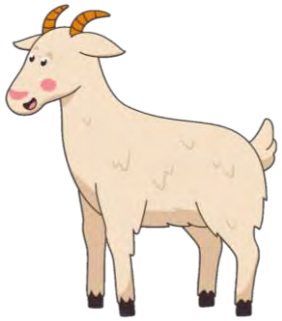
Biology of Reproduction, 2022, 1–14
https://doi.org/10.1093/biolre/ioac188
Review
OXFORD

Molecular insights to the sperm–cervix interaction and the consequences for cryopreserved sperm†

Sophie Warr[1,*], Taylor Pini[2], Simon P. de Graaf[1] and Jessica P. Rickard[1]



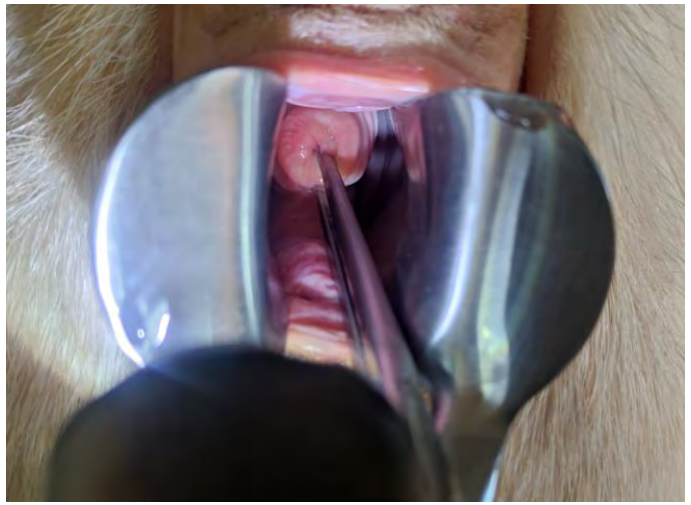
AI techniques in goats



Transcervical

most widespread

Cervical immobilization



Embrapa AI technique

Reproductive Biology 17 (2017) 268–273

Contents lists available at ScienceDirect

Reproductive Biology

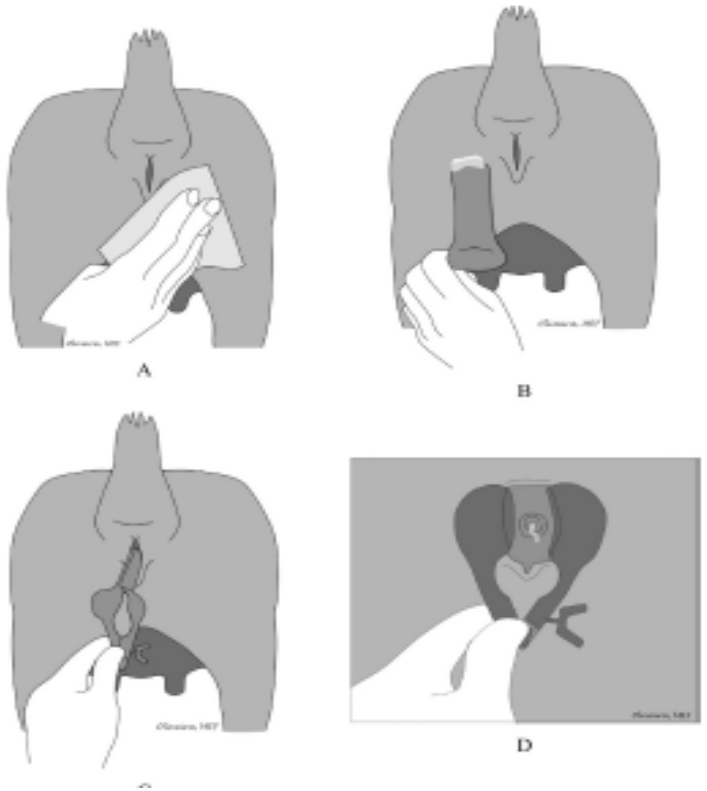
journal homepage: www.elsevier.com/locate/repbio

Original article

Reproductive features and use of an anti-inflammatory drug in estrus-induced dairy goats artificially inseminated in a standing position with cervix immobilization

Jeferson Ferreira Fonseca[a,*], Gilmar Pereira Alvim[b], Joanna Maria Gonçalves Souza-Fabjan[c,d], Maria Emília Franco Oliveira[e], Viviane Lopes Brair[d], Felipe Zandonadi Brandão[c], Olivardo Facó[a]

Embrapa AI technique



Intrauterine AI was more frequent (P<0.01) in Plu (**100%**; 32/32) than in Null (70%; 23/33)

Cervical transposing was more time-consuming (P<0.01) in **Null (44 s) than Plu (21 s)**

Table 2

Pregnancy rates (%) according to the local of semen deposition in estrus-induced Saanen goats artificially inseminated (AI) 51–54 h after sponge removal and receiving 1 mL saline (CONTROL) or 50 mg Flunixin-Meglunine (FLUNIXIN) i.m. at the time of AI.

Depth (cm)	CONTROL	FLUNIXIN	Total	P value
0 cm (0/pericervical)[1]	50.0 (1/2)	0.0 (0/3)	20.0 (1/5)	–
1–2 cm (1/Superficial cervical)[1]	0.0 (0/1)	0.0 (0/1)	0.0 (0/2)	–
2–3 cm (2/Initial cervical)[1]	100.0 (1/1)	0.0 (0/1)	50.0 (1/2)	–
3–4 cm (3/Intermediate cervical)[1]	100.0 (1/1)	0.0 (0/0)	100.0 (1/1)	–
5 cm (5/Uterine)[1]	62.9[a] (17/27)	35.7[b] (10/28)	49.1 (27/55)	0.043
Total	62.5[a] (20/32)	30.3[b] (10/33)	46.1 (30/65)	0.009

() Number of females pregnant/Number of females inseminated.

[a,b]Percentages with different superscripts within a row differed (Chi-Square test).

[1] Cervical rings surpassed/locale of semen deposition.

Embrapa AI technique cervical mucus

Reproductive Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Reproductive Biology

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Original article

Evaluation of cervical mucus and reproductive efficiency of seasonally anovular dairy goats after short-term progestagen-based estrous induction protocols with different gonadotropins

Jeferson F. Fonseca[a,*], Joanna M.G. Souza-Fabjan[b], Maria Emilia F. Oliveira[c], Renata C. Cruz[d], Luciana V. Esteves[b], Maria Pia S.L. Matos de Paiva[e], Felipe Z. Brandão[b], Antônio B. Mancio[d]

Table 3
Intervals (mean ± SD) from first detection of different cervical mucus types to the onset of behavioral estrus or ovulation time in Toggenburg goats subjected to the estrous induction protocols with a 6-day progestagen treatment plus d-cloprostenol and different gonadotropins administered 24 h before sponge removal (Experiment 1).

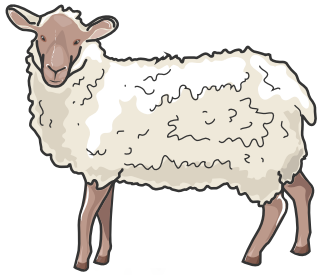
Cervical mucus (types)	Time (hours)	
	Estrous onset	Ovulation time
Crystalline (1)	−9.6 ± 10.5	−44.8 ± 16.9
Crystalline/striated (2)	−2.0 ± 8.0	−34.8 ± 1.5
Striated (3)	13.9 ± 7.7	−18.5 ± 7.7
Striated/caseous (4)	30.0 ± 10.3	−1.9 ± 6.8
Caseous (5)	41.3 ± 14.6	9.1 ± 9.8

The negative values indicate that the specific type of cervical mucus was observed before the onset of estrus or ovulation. P > 0.05.

Fig. 2. Observations on the cervical mucus discharge in the cervical os of the goats. 0–no discharge, no estrus; 1–crystalline mucus; 2–crystalline/striated mucus; 3–striated mucus; 4–striated/caseous mucus; and 5–caseous mucus. See the text for details of mucous categories.

Fig. 3. Observations on the cervical mucus discharge in the vulva of the goats. 0–no discharge, no estrus; 1–crystalline mucus; 2–crystalline/striated mucus; 3–striated mucus; 4–striated/caseous mucus; and 5–caseous mucus. See the text for details of mucous categories.

Cervical mucus



Tropical Animal Health and Production (2021) 53:223
https://doi.org/10.1007/s11250-021-02667-6

SHORT COMMUNICATIONS

Vaginal cytology and cervical mucus as tools to predict ovulation time in small ruminants

Joanna Maria Gonçalves Souza-Fabjan[1] · Viviane Lopes Brair[1] · Dafne dos Santos Silva[2] · Ana Paula Pereira Schmidt[1] · Lucas Machado Figueira[1] · Paulo Sérgio Cerqueira Rangel[2] · Gabriel Brun Vergani[3] · Vitória de Oliveira Machado[4] · Maria Emilia Franco Oliveira[3] · Jeferson Ferreira da Fonseca[5]

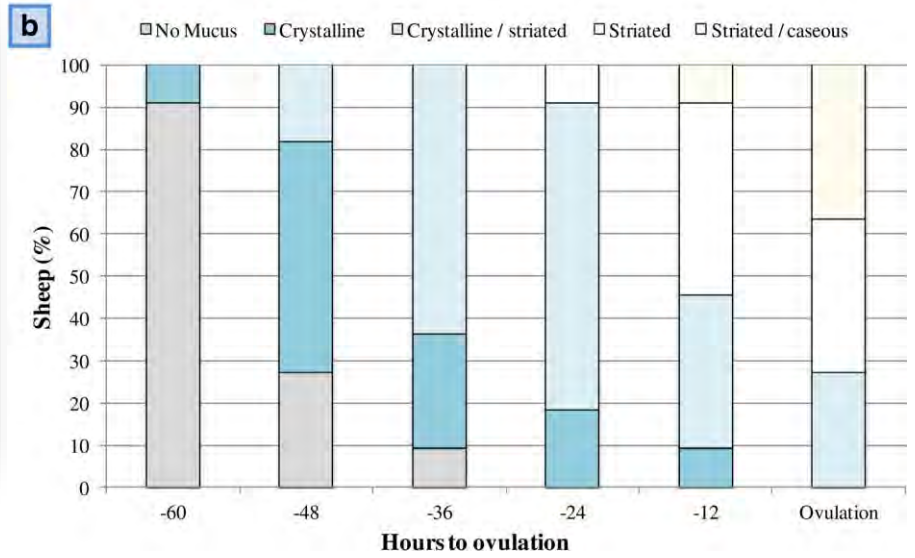


Fig. 1 Cervical mucus types of Santa Inês ewes hormonally induced to estrus.

Pregnancy rates – Embrapa AI technique

Research Centers

Reproductive Biology 17 (2017) 268–273

Contents lists available at ScienceDirect

Reproductive Biology

journal homepage: www.elsevier.com/locate/repbio

Original article

Reproductive features and use of an anti-inflammatory drug in estrus-induced dairy goats artificially inseminated in a standing position with cervix immobilization

Jeferson Ferreira Fonseca[a,*], Gilmar Pereira Alvim[b], Joanna Maria Gonçalves Souza-Fabjan[c,d], Maria Emília Franco Oliveira[e], Viviane Lopes Brair[d], Felipe Zandonadi Brandão[c], Olivardo Facó[a]

P4

62 %

Animal Reproduction Science 209 (2019) 106172

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effects of d-cloprostenol administrations with 7.5 and 11.5-day intervals between administrations on pregnancy rates after artificial insemination in estrous cyclic dairy goats

G.C. Bonato[a], A.L.R.S. Maia[b], L.R. Côrtes[b], T.A. Oliveira[c], A.M. Arrais[d], L.M. Figueira[e], M.E.F. Oliveira[f], J.M.G. Souza-Fabjan[b], J.F. Fonseca[a,g,*]

PG F

66 to 80%

Animal Reproduction Science 221 (2020) 106571

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effect of a 12-h increment in the short-term treatment regimen on ovarian status, estrus synchrony, and pregnancy rate in artificially inseminated dairy goats

Cleber Jonas Carvalho-de-Paula[a], Joanna Maria Gonçalves Souza-Fabjan[a], Joedson Dantas Gonçalves[b], Jenniffer Hauschildt Dias[c], Guilherme Nunes de Souza[a,d], Maria Emilia Franco Oliveira[b,e], Jeferson Ferreira Fonseca[c,e,*]

P4

68 %

Animal Reproduction Science 181 (2017) 16–23

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

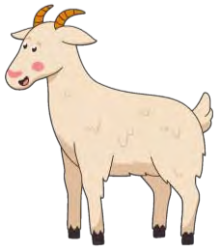
Reproductive parameters of dairy goats after receiving two doses of d-cloprostenol at different intervals

A.L.R.S. Maia[a], F.Z. Brandão[a], J.M.G. Souza-Fabjan[a,b], M.F.A. Balaro[a], M.E.F. Oliveira[c], O. Facó[d], J.F. Fonseca[d,*]

PG F

85 to 93%

Results of our AI technique in commercial systems



Anais do XXIII Congresso Brasileiro de Reprodução Animal (CBRA-2019); Gramado, RS, 15 a 17 de maio de 2019.

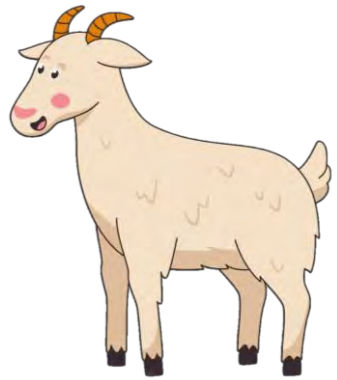
Recent advances in goat artificial insemination in Brazil

Recentes avanços da inseminação artificial de caprinos no Brasil

Jeferson Ferreira da Fonseca[1, £], Vitória de Oliveira Machado[2], Maria Pia S.L. Matos de Paiva[3], Olivardo Facó[1], Joanna Maria Gonçalves Souza-Fabjan[4]

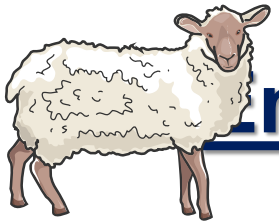
50-70% of pregnancy rate after 1 AI with F/T semen in dairy goats.

(n= > 2500 goats)



AI in 60 goats, in <2h

Photo provided by Jeferson Fonseca

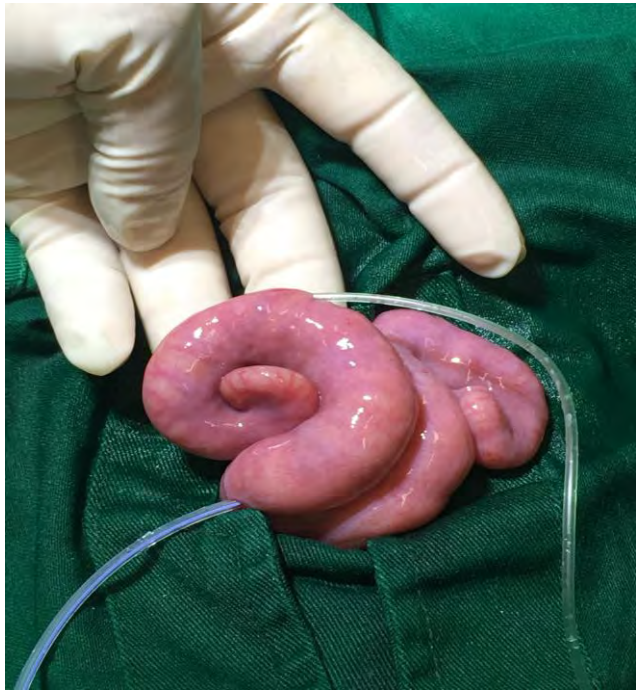


Embryo collection techniques in small ruminants

most widespread

Laparoscopy

Laparotomy

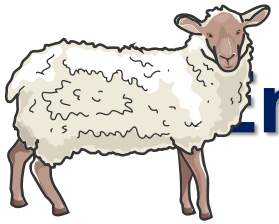


South African Journal of Animal Science 2009, 39 (Supplement 1) 193
©South African Society for Animal Science
Peer-reviewed paper: 10th World Conference on Animal Production

Can repeated superovulation and embryo recovery in Boer goats limit donor participation in a MOET programme?

K.C. Lehloenya[1#], J.P.C. Greyling[2] and S. Grobler[3]

Labor-Intensive



Embryo collection techniques in small ruminants

Non-surgical embryo recovery (NSER)

Nonsurgical embryo collection in goats treated with prostaglandin F2alpha and oxytocin

R. J. Pereira, B. Sohnrey and W. Holtz

J Anim Sci 1998. 76:360-363.

Available online at www.sciencedirect.com
ScienceDirect
Theriogenology
ELSEVIER
Theriogenology 69 (2008) 197–203
www.theriojournal.com

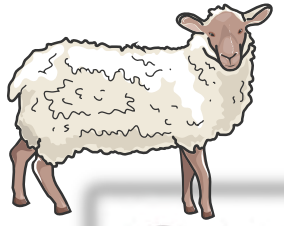
Repeat superovulation, non-surgical embryo recovery, and surgical embryo transfer in transgenic dairy goats

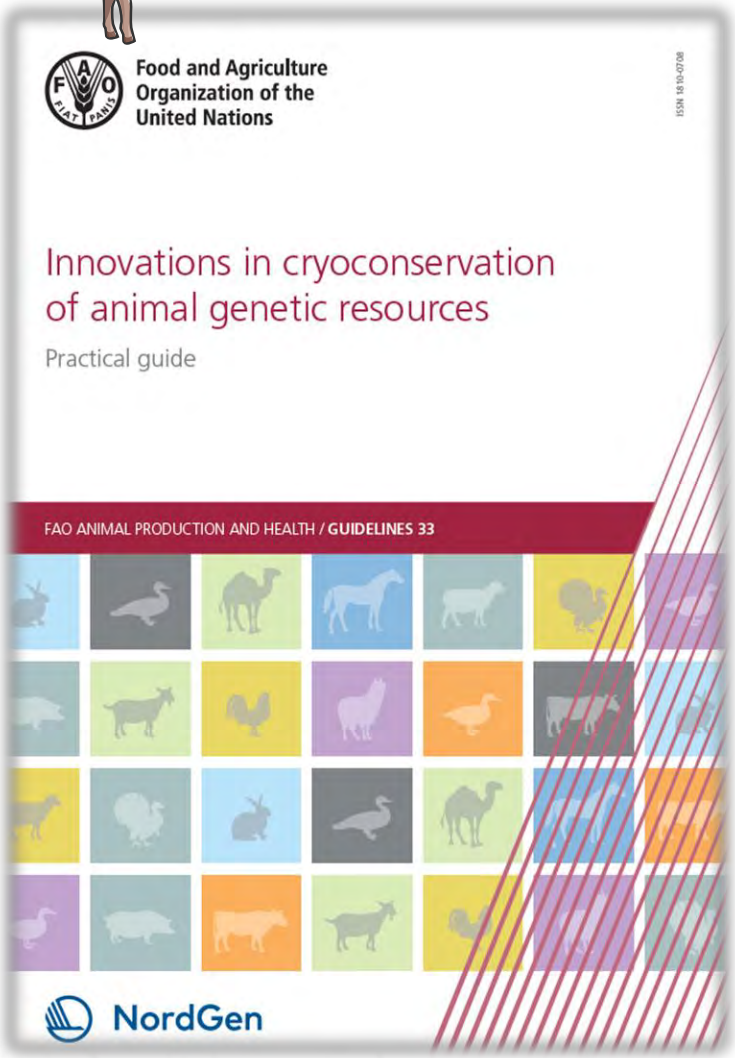
D. Melican *, W. Gavin

Estradiol-17β-Oxytocin-Induced Cervical Dilation in Sheep: Application to Transcervical Embryo Transfer[1,2]

Meghan C. Wulster-Radcliffe, Beth A. Costine, and Gregory S. Lewis[3]

Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061-0306





Food and Agriculture Organization of the United Nations
Rome, 2023

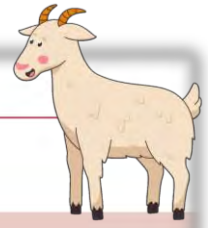


TABLE 6.1
Comparison of non-surgical versus surgical embryo collection in livestock species

Characteristic	Species				
	Cattle	Sheep	Goats	Pigs	Horses
Non-surgical					
ease[a]	1	5	3	3	1
percent of treated females with ≥ 1 embryo per collection	85	> 50	> 70	< 35	80
transferable embryos per collection (*n*)	4–8	0–8	3–8	0–5	≤ 1
collections per year (*n*)[b]	3–6	3–6	3–6	2–4	4–6
recommended for use?	Yes	No	Yes	No	Yes
Surgical					
easea	5	2	2	1	4
percent of treated females with ≥ 1 embryo per collection	85	> 70	> 70	95	< 80
transferable embryos per collection (*n*)	4–8	3–8	3–8	10–25	≤ 1
collections per year (*n*)[b]	3	1–2	1–2	2	3
post-surgical adhesions	+++	++++	++++	++++	+
recommended for use?	No	Yes	No	Yes	No[c]

Note: Ranges presented are estimated from multiple scientific and in-field sources. The values are based on the use of superovulated donors, technicians with the appropriate expertise, and optimal donor nutrition and animal management practices.

[a] Ease of the procedure, with 1 being the easiest and 5 being the most difficult to perform.

[b] Post-surgical adhesions generally dictate the number of surgical collections per female during her lifetime. The number of surgeries per female may be designated by governmental regulations and/or an institutional review board.

[c] Frozen–thawed equine embryos > 300 µm in diameter rarely produce a pregnancy following transfer.

Source: Authors' own elaboration.

CSIRO PUBLISHING

Reproduction, Fertility and Development, 2019, **31**, 1–10
https://doi.org/10.1071/RD18324

Non-surgical embryo transfer in goats and sheep: the Brazilian experience

Jeferson F. Fonseca[A,F], *Maria Emilia F. Oliveira*[B], *Felipe Z. Brandão*[C], *Ribrio I. T. P. Batista*[C], *Alexandre R. Garcia*[D], *Pawel M. Bartlewski*[E] *and Joanna M. G. Souza-Fabjan*[C]

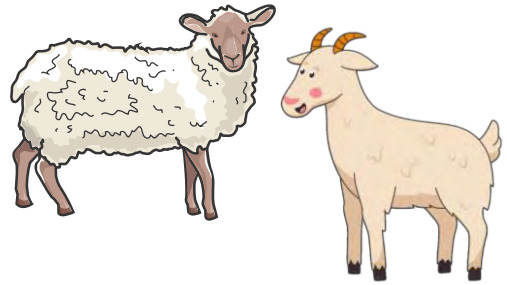
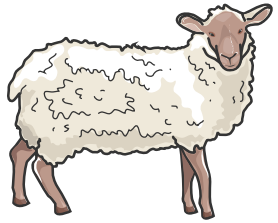


Table 3. Comparisons among embryo recovery techniques in goats and sheep in relation to the main steps related to techniques

Procedure	Laparotomy	Laparoscopy	Transcervical
Animal fasting	>24 h	>24 h	Not needed
Sedation	Deep	Deep	Superficial
Animal position during the procedure	Dorsal recumbence	Dorsal recumbence	Four leg station
Anaesthesia	General	General	Epidural or local
Laparoscopic equipment	Needed	Needed	Not needed
Ultrasound equipment	Not needed	Not needed	Recommended for CL count
Embryo recovery efficiency	Precise	Precise	Imprecise without CL count
Fluid recovery efficiency	High (>90%)	–	High (>90%)
Animal return to basal physiological conditions	Long (6 h)	Long (6 h)	Low (minutes)
Sequelae in the reproductive tract	High	Intermediate	Low
Minimal time between successive flushings	>30 days	>30 days	1 week
Effective number of flushings per donor	3	–	6
Animal welfare	Low	Intermediate	High
Technician skill needed	Intermediate	High	High
Total cost	High	High	Low



Donor selection

Primary criteria for selecting donors for MOET

- Genetic/economic value
- Responsiveness to SOV

Theriogenology 113 (2018) 146–152

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

ELSEVIER

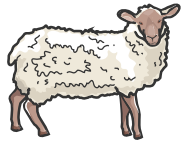
THERIOGENOLOGY

Anti-Müllerian hormone and antral follicle count are more effective for selecting ewes with good potential for in vivo embryo production than the presence of *FecG*^{E} mutation or eCG pre-selection tests

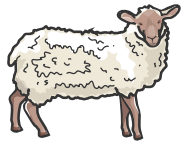
Pedro Henrique Nicolau Pinto [a, *], Mario Felipe Alvarez Balaro [a], Joanna Maria Gonçalves Souza-Fabjan [a], Lilian dos Santos Ribeiro [a], Gláucia Mota Bragança [a], Ceci Ribeiro Leite [a], Eduardo Kenji Nunes Arashiro [a], Kleibe de Moraes Silva [b], Jeferson Ferreira Da Fonseca [c], Felipe Zandonadi Brandão [a]

For NSER

- Avoid nulliparous
- BCS should be intermediate (not too thin, but not obese)
- Post-partum ewes between 100 and 150 days



Donor selection



PhD Student: Juliana Dantas

PhD student: Lucas Figueira

Received: 21 May 2018 | Revised: 31 July 2018 | Accepted: 2 August 2018
DOI: 10.1111/rda.13306

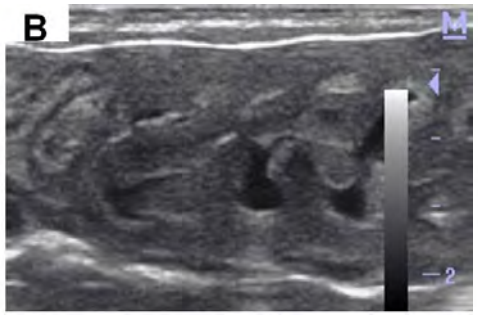
SHORT COMMUNICATION

WILEY Reproduction in Domestic Animals

Cervical transposition test using Hegar dilator at oestrus as a tool to select ewes for transcervical embryo collection

Juliana Dantas Rodrigues Santos[1] | Eduardo Kenji Nunes Arashiro[1] | Mário Felipe Alvarez Balaro[1] | Joanna Maria Gonçalves Souza-Fabjan[1] | Pedro Henrique Nicolau Pinto[1] | Clara Vieira de Souza[1] | Ceci Ribeiro Leite[1] | Jeferson Ferreira da Fonseca[2] | Felipe Zandonadi Brandão[1]

Sensibility: 85.7%; Specificity: 66.6%; Accuracy: 80%



Cervix rectilinear, intermediate, and highly asymmetrical

Received: 22 June 2020 | Accepted: 7 September 2020
DOI: 10.1111/rda.13825

ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

Ultrasonographic cervical evaluation: A tool to select ewes for non-surgical embryo recovery

Lucas Machado Figueira[1,2] | Nadja Gomes Alves[1] | Joanna Maria Gonçalves Souza-Fabjan[2] | Gabriel Brun Vergani[3] | Maria Emilia Franco Oliveira[3,4] | Renato Ribeiro de Lima[1] | Jeferson Ferreira Fonseca[4]

PhD student: Jenniffer Dias

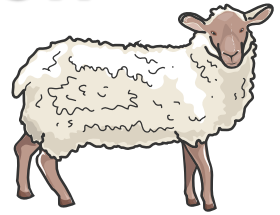
Small Ruminant Research 220 (2023) 106914

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Donor selection



Different doses of pFSH are effective to promote follicular growth, superovulatory response, and embryo yield in White Dorper ewes

J.H. Dias [a], G.B. Vergani [b], J.D. Gonçalves [b], T.A. Oliveira [c], J.M. Penitente-Filho [a], V.S.A. Pereira [d], S.N. Esteves [d], A.R. Garcia [d], R.I.T.P. Batista [c], M.E.F. Oliveira [b], J.M.G. Souza-Fabjan [c], J.F. Fonseca [e,*]

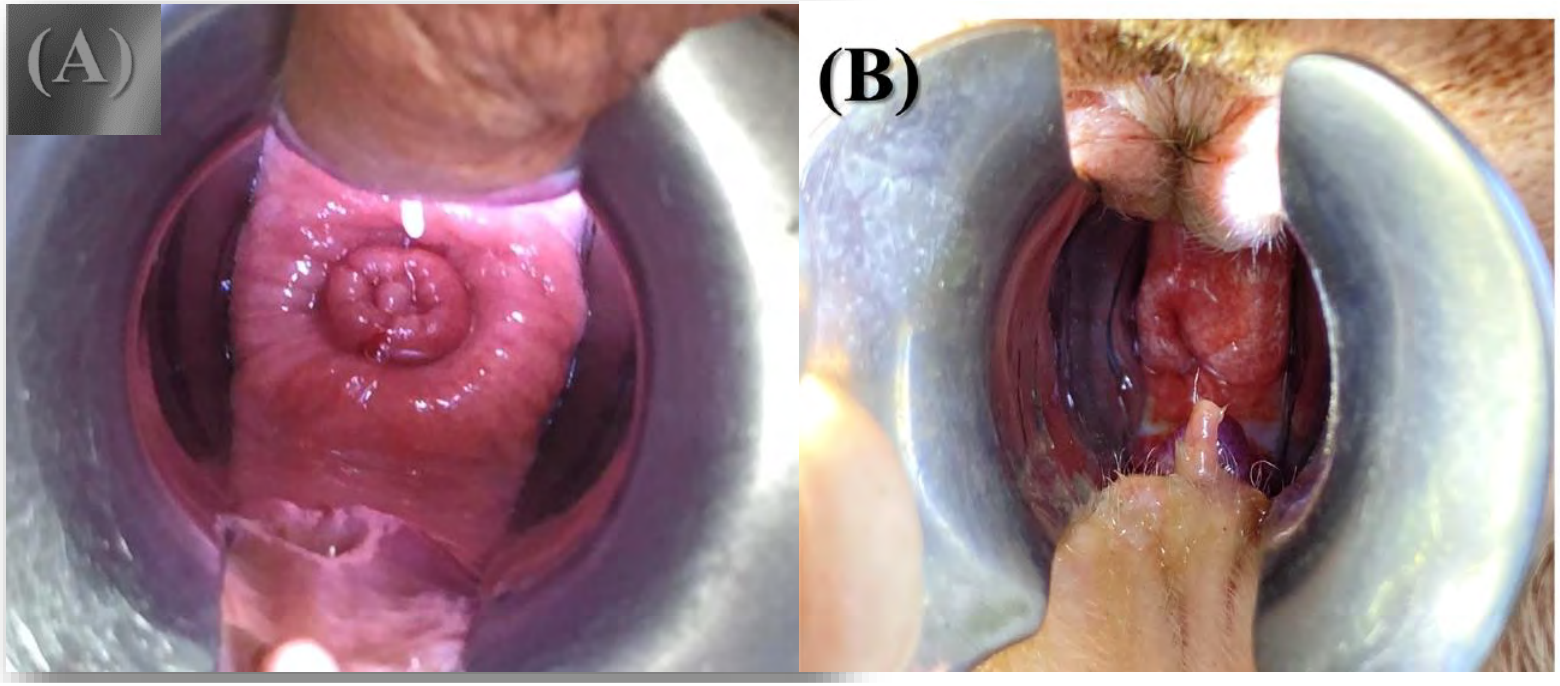
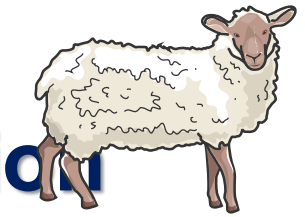


Fig. 4. Vaginoscopy in ewes showing total vaginal speculum introduction and complete cervical ostium visualization (A). Vaginal speculum positioned into vestibulo before hymenal prega (B).

Hormonal protocol: Synchronization/Superovulation



Theriogenology 103 (2017) 24–29

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of different hormonal combinations on follicular wave emergence and superovulatory response in sheep

Joanna Maria Gonçalves Souza-Fabjan [a, b, *], Rômulo Mendonça da Rosa [a], Mário Felipe Alvarez Balaro [a], Pedro Henrique Nicolau Pinto [a], Gustavo Bervian dos Santos [a], Eduardo Kenji Nunes Arashiro [a], Jeferson Ferreira da Fonseca [c], Rodolfo Ungerfeld [d], Felipe Zandonadi Brandão [a, *]

Small Ruminant Research 220 (2023) 106914

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Different doses of pFSH are effective to promote follicular growth, superovulatory response, and embryo yield in White Dorper ewes

J.H. Dias [a], G.B. Vergani [b], J.D. Gonçalves [b], T.A. Oliveira [c], J.M. Penitente-Filho [a], V.S.A. Pereira [d], S.N. Esteves [d], A.R. Garcia [d], R.I.T.P. Batista [c], M.E.F. Oliveira [b], J.M.G. Souza-Fabjan [c], J.F. Fonseca [e, *]

Animal Reproduction Science 247 (2022) 107101

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Single dose of 300 IU hCG in the early luteal phase in superovulated ewes: Effects on corpora lutea, progesterone profile, and embryo recovery

J.H. Dias [a], J.D. Gonçalves [b], A.M. Arrais [c], R.I.T.P. Batista [d], J.M.G. Souza-Fabjan [d], R. Bastos [e], L.G.B. Siqueira [f], M.E.F. Oliveira [b], J.F. Fonseca [g, *]

Theriogenology 181 (2022) 140–146

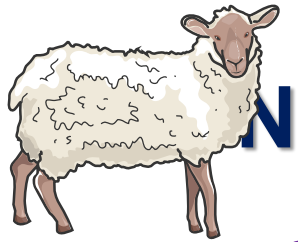
Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

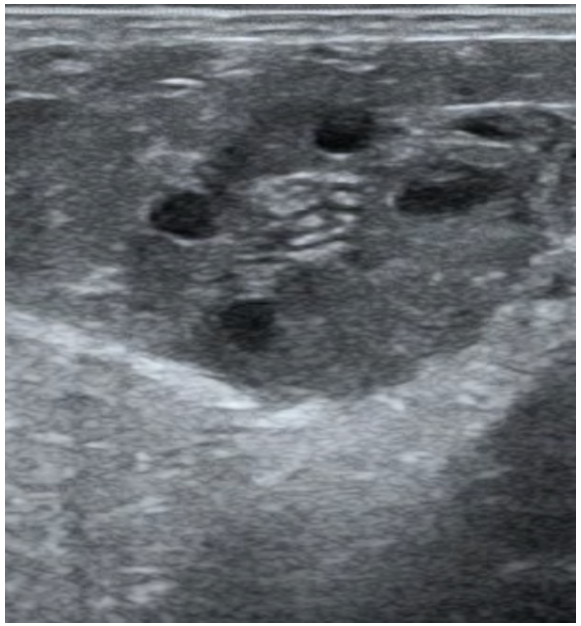
Biostimulation with the ram effect increases the follicle recruitment, ovulatory diameter, and embryo viability rate in superovulated ewes

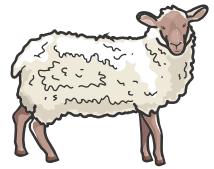
Augusto Ryonosuke Taira [a, *], Felipe Zandonadi Brandão [a], Viviane Lopes Brair [a], Isabel Oliveira Cosentino [a], Felipe Seabra Cardoso Leal [a], Ana Clara Sarzedas Ribeiro [a], Mário Felipe Alvarez Balaro [a], Ribrio Ivan Tavares Pereira Batista [a], Joanna Maria Gonçalves Souza-Fabjan [a], Jeferson Ferreira da Fonseca [b], Rodolfo Ungerfeld [c]



Non-invasive method to assess SOV responses

Can we replace the pre-collection laparoscopy by an US evaluation?





n= 75 ewes; n= 150 ovaries

PhD student: Pedro Pinto

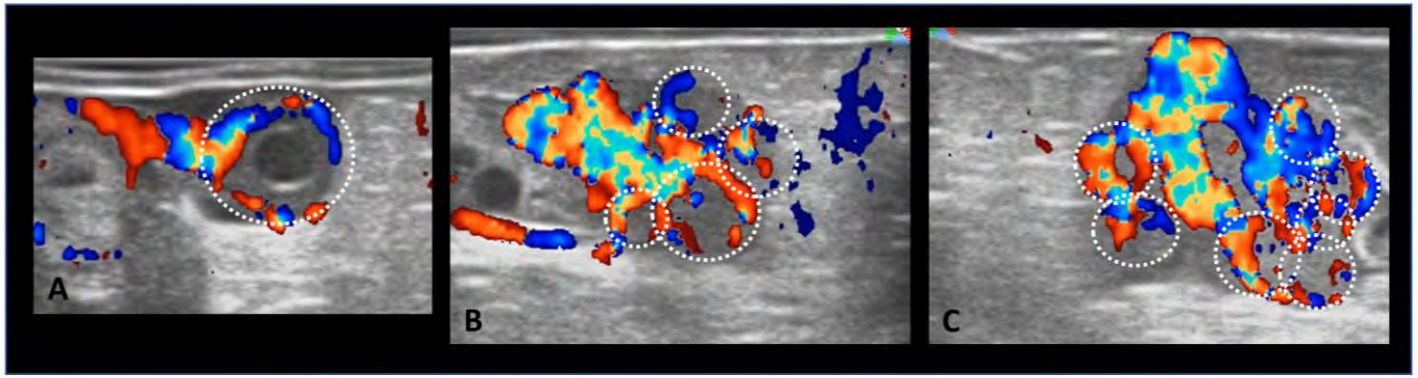
Received: 11 July 2017 | Accepted: 4 September 2017
DOI: 10.1111/rda.13089

SHORT COMMUNICATION

WILEY Reproduction in Domestic Animals

Colour-Doppler ultrasound imaging as a laparoscopy substitute to count corpora lutea in superovulated sheep

PHN Pinto[1] | GM Bragança[1] | MFA Balaro[1] | EKN Arashiro[1] | GB dos Santos[1] | GN de Souza[2] | JMG Souza-Fabjan[1] | JF Da Fonseca[3] | FZ Brandão[1]



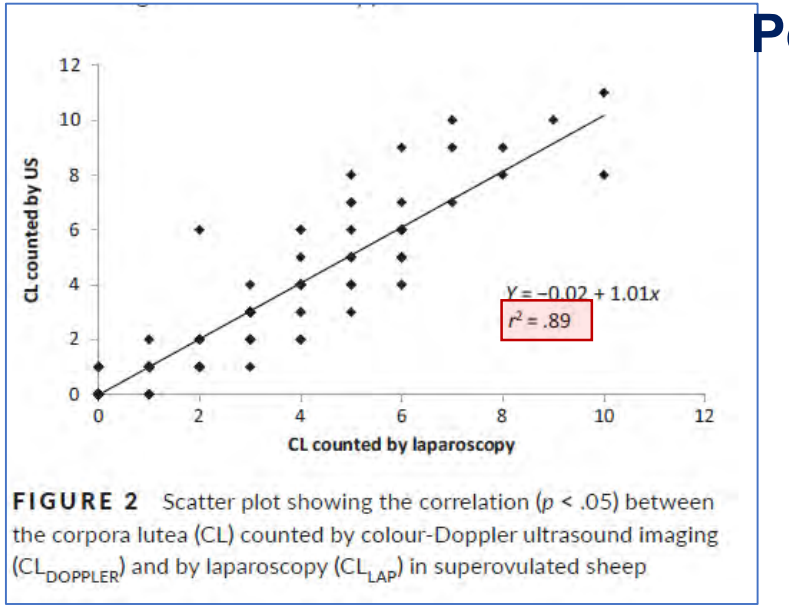
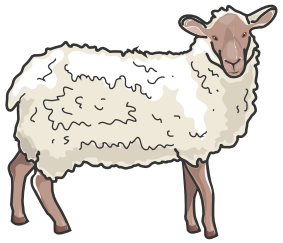


FIGURE 2 Scatter plot showing the correlation ($p < .05$) between the corpora lutea (CL) counted by colour-Doppler ultrasound imaging ($CL_{DOPPLER}$) and by laparoscopy (CL_{LAP}) in superovulated sheep

CL	SENS	SPEC	VPP	VPN	Kappa	ACR
1	0.98	0.93	0.91	0.99	0.95	0.89
2	0.98	0.94	0.89	0.99	0.95	0.84
3	0.95	0.95	0.88	0.98	0.95	0.75
4	0.86	0.96	0.83	0.97	0.94	0.67
5	0.67	0.96	0.74	0.95	0.92	0.61

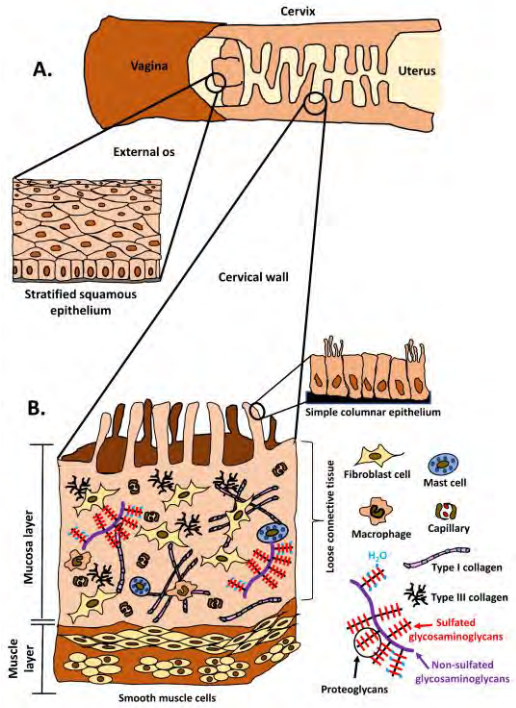
CURRENTLY...

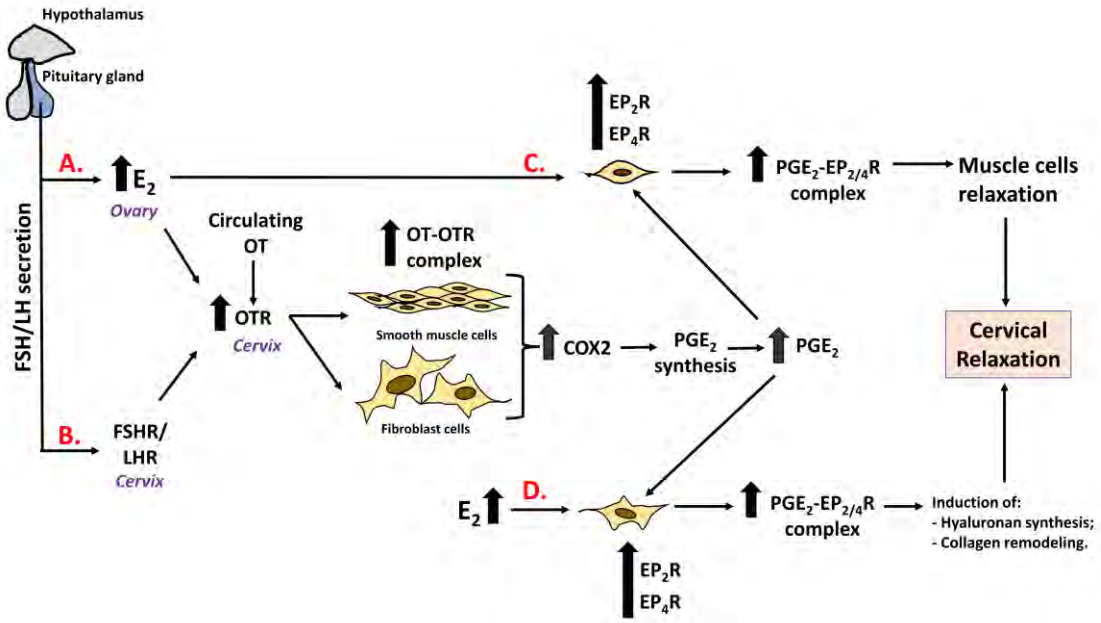


SO V

Cervical dilation treatment

- Need for cervical dilation before embryo recovery, especially in sheep
- The hormonal protocols were based on the mechanisms of natural cervical ripening (estrus and pre-parturition)





Review under preparation (Dias et al.)

Table 3. Studies conducted in goats that were subjected to **hormonal cervical dilation protocols** prior to NSER.

Breed	Category	Hormonal Protocol	Efficiency (%)		Reference
			Cervical penetration*	Embryo recovery**	
Canindé	Multiparous	37.5 µg d-cloprostenol i.m. (−16 h to NSER)	100 (11/11)	35 (37/106)	Fonseca et al. (2021)
Moxotó	Multiparous		100 (12/12)	53 (88/167)	
Saanen	Multiparous	37.5 µg d-cloprostenol i.m. (−12 h to NSER)	100 (21/21)	71 (121/170)	Maia et al. (2020)
Saanen	Multiparous	30 µg d-cloprostenol i.m. (−12 h to NSER)	100 (10/10)	ND	Fonseca et al. (2013)
Toggenburg	Multiparous	125 µg cloprostenol i.m. (−24 h to NSER)	100 (18/18)	ND	Amorim et al. (2011)
Saanen	Multiparous	50 µg cloprostenol i.m. (−24 h to NSER)	62 (8/13)	53 (50/94)	Lima-Verde et al. (2003)
Boer	Multiparous	5 mg dinoprost i.m. (−16 h) + 1 IU OT i.v. (−0 h)	100 (7/7)	91 (82/90)	Pereira et al. (1998)
		5 mg dinoprost i.m. (−8 h) + 1 IU OT i.v. (−0 h)	100 (6/6)	91 (48/53)	
		5 mg dinoprost i.m. (−0 h) + 1 IU OT i.v. (−0 h to NSER)	100 (6/6)	52 (28/54)	

Abbreviations: NSER = non-surgical embryo recovery; ND: not determined; OT = oxytocin; i.m. = intramuscular; i.v. = intravenous.
***Donors successfully penetrated and flushed x 100/donors with corpora lutea at NSER.**
****Number of total structures recovered x 100/number of corpora lutea per flushed female.**

In goats, only PGF

Vet Record

ORIGINAL RESEARCH

Veterinary Record: first published as 10.1136/vr.105906

Embryo development is impaired in goats that are treated for hydrometra and subsequently subjected to superovulation

Ana Lucia R S Maia [1], Aline M Arrais [2], Lucia Prellwitz [1], Ribrio I T P Batista [1], Lucas M Figueira [3], Lucas F L Correia [1], Jeferson F Fonseca [4], Joanna M G Souza-Fabjan [1]

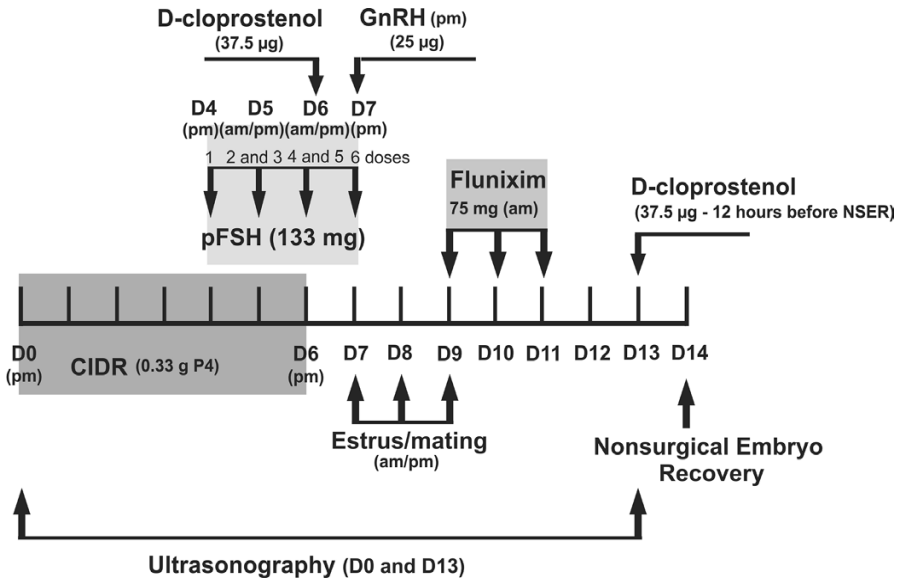


Table 2 Parameters of healthy (control) or hydrometra-treated goats submitted to oestrus induction and superovulation, followed by non-surgical embryo recovery (mean±SEM)

Parameters	Control (n=11)	Hydrometra* (n=10)	P value
Goats in oestrus (%)	100.0	100.0	1.00
Interval to oestrus (h)	29.1±3.9	31.0±2.3	0.55
Duration of oestrus (h)	21.8±2.0	18.2±2.8	0.25
Number of mating	2.8±0.2	2.5±0.3	0.29
Number of corpora lutea (CL)	8.5±1.3 (93)	7.7±0.9 (77)	0.65
Responsive donors (≥3 CL) (%)	91.0 (10/11)	100.0 (10/10)	1.00
Cervical transposition and uterine flushing (%)	100.0	100.0	1.00
Collection duration (min)	23.2±1.9	19.1±0.6	0.08
Recovery rate (%)†	81.7 (76/93)	58.4 (45/77)	0.01
Recovered structures per goat	6.9±1.7	4.5±1.2	0.37
Viable rate (%)	71.0 (54/76)	60.0 (27/45)	0.23
Viable embryos per goat	4.9±1.6	2.7±0.9	0.23

() Number of total CL, number of animals or number of structures.
*Animals detected with hydrometra were treated with three doses of prostaglandin F2a and presented no uterine fluid for two months before the start of this experiment.
†Total of structures/CLx100.

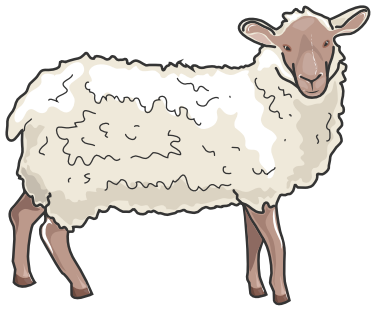
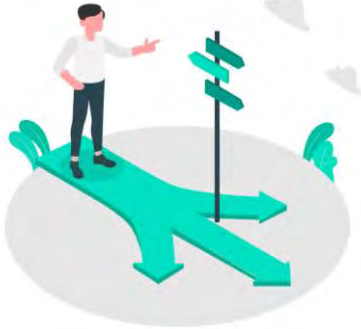


Table 2. Studies conducted in sheep that were subjected to **hormonal cervical dilation protocols** prior to NSER.

Breed	Parity	Hormonal Protocol	Efficiency (%)		Reference
			Cervical penetration*	Embryo recovery**	
Crossbreed (Lacaune x Santa Inês)	Diverse	1 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	64 (9/14)	83 (80/97)	Dias et al. (2023)
		0.5 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	83 (10/12)	107 (89/83)	
		0.0 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	90 (9/10)	60 (55/91)	
Morada Nova	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	86 (25/29)	71 (176/248)	Oliveira et al. (2022)
Morada Nova	Multiparous		94 (15/16)	99 (135/136)	
Santa Inês	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	90 (17/19)	83 (209/252)	Fonseca et al. (2021)
Somalis	Multiparous		94 (17/18)	57 (101/178)	
Morada Nova	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	94 (16/17)	60 (95/159)	Arrais et al. (2021)
Santa Inês	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	84 (26/31)	ND	Oliveira et al. (2020)
Lacaune	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−10 h) and 50 IU OT i.v. (−20 min to NSER)	94 (31/33)	61 (187/306)	Figueira et al. (2020c)
Lacaune	Diverse	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	89 (32/36)	62 (143/232)	Figueira et al. (2020b)
Dorper	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	100 (12/12)	40 (8/20)	Dias et al. (2020)
		0.5 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	92 (11/12)	39 (9/23)	
		0.0 mg EB i.m. + 37.5 µg d-cloprostenol i.m. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	83 (10/12)	53 (10/19)	
Santa Inês	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	57 (12/21)	ND	Prellwitz et al. (2019)
		1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v.g. (−20 min to NSER)	57 (12/21)	ND	
Santa Inês	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	82 (9/11)	ND	Fonseca et al. (2019d)
		1 mg EB i.v.g. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	80 (8/10)	ND	
Santa Inês	Multiparous	1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	78 (7/9)	ND	Fonseca et al. (2019c)
		1 mg EC i.m. + 37.5 µg d-cloprostenol i.v. (−16 h) and 50 IU OT i.v. (−20 min to NSER)	44 (4/9)	ND	
		1 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−10 h) and 50 IU OT i.v. (−20 min to NSER)	39 (5/13)	ND	
		0.0 mg EB i.m. + 37.5 µg d-cloprostenol i.v. (−10 h) and 50 IU OT i.v. (−20 min to NSER)	27 (3/11)	ND	
Santa Inês	Multiparous	200 µg misoprostol i.v.g. (PGE1 analogue) (−5 h to NSER)	67 (20/30)	ND	Leite et al. (2018)
		100 IU OT i.v. (−15 min) + 100 µg EB i.v.	90 (27/30)	ND	
		200 µg misoprostol i.v.g. + 100 IU OT i.v. (−15 min) and 100 µg EB i.v. (−12 h to NSER)	83 (25/30)	ND	
Dorper	Multiparous	200 µg misoprostol i.v.g. (−5 h to NSER)	95 (55/58)	ND	Gusmão et al. (2009)

Abbreviations: NSER = non-surgical embryo recovery; ND = not determined; EB = estradiol benzoate; EC = estradiol cypionate; OT = oxytocin; PGE = prostaglandin E; i.m. = intramuscular; i.v. = intravenous; i.v.g. = intravaginal; l.v. = inlaterovulvar.
*Donors successfully penetrated and flushed x 100/donors with corpora lutea at NSER (%).
**Number of total structures recovered x 100/ number of corpora lutea per flushed female (%).

Cervical dilation treatments



PhD Student: Fabiana Zambrini

Animal Reproduction Science 203 (2019) 25–32

Contents lists available at ScienceDirect

Animal Reproduction Science

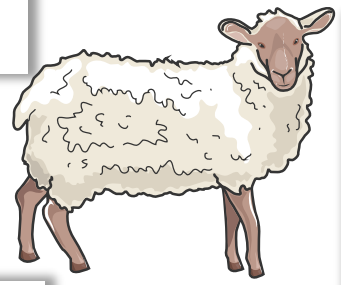
journal homepage: www.elsevier.com/locate/anireprosci

Cervical penetration rates and efficiency of non-surgical embryo recovery in estrous-synchronized Santa Inês ewes after administration of estradiol ester (benzoate or cypionate) in combination with d-cloprostenol and oxytocin

J.F. Fonseca[a,*], F.N. Zambrini[b], J.D. Guimarães[b], M.R. Silva[c], M.E.F. Oliveira[d], P.M. Bartlewski[e], J.M.G. Souza-Fabjan[f]

EB i.m. versus saline i.m.

EB i.m. versus ECP i.m.



Received: 11 July 2018 | Accepted: 16 August 2018

DOI: 10.1111/rda.13318

ORIGINAL ARTICLE

WILEY Reproduction in Domestic Animals

Combined treatment with oestradiol benzoate, d-cloprostenol and oxytocin permits cervical dilation and nonsurgical embryo recovery in ewes

Jeferson Ferreira da Fonseca[1] | Fabiana Nunes Zambrini[2] | José Domingos Guimarães[2] | Marcio Roberto Silva[3] | Maria Emilia Franco Oliveira[4] | Felipe Zandonadi Brandão[5] | Pawel Mieczyslaw Bartlewski[6] | Joanna Maria Gonçalves Souza-Fabjan[5]

EB i.m. versus ivg.

Received: 2 May 2019 | Revised: 2 June 2019 | Accepted: 21 June 2019

DOI: 10.1111/rda.13499

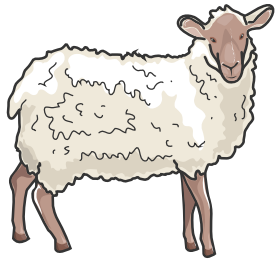
ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

Comparison of the intravenous and intravaginal route of oxytocin administration for cervical dilation protocol and non-surgical embryo recovery in oestrous-induced Santa Inês ewes

Lucia Prellwitz[1] | Fabiana Nunes Zambrini[2] | José Domingos Guimarães[2] | Marco Antonio Paula de Sousa[3] | Maria Emília Franco Oliveira[4] | Alexandre Rosetto Garcia[5] | Sérgio Novita Esteves[5] | Pawel Mieczyslaw Bartlewski[6] | Joanna Maria Gonçalves Souza-Fabjan[1] | Jeferson Ferreira Fonseca[7]

OT ivg. versus iv.



Our "*current*" cervical dilation protocol

International Embryo Technology Society
45th Annual Conference
Sheraton New Orleans Hotel
New Orleans, Louisiana
January 20–23, 2019

18TH INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION

JUNE 26–30TH 2016
LE VINCI IN TOURS FRANCE
The cutting edge science... in the garden of France.
Reproductive physiology, pathology and biotechnologies in domestic and wild animal species.

CSIRO PUBLISHING

Reproduction, Fertility and Development, 2019, **31**, 1–10
https://doi.org/10.1071/RD18324

Non-surgical embryo transfer in goats and sheep: the Brazilian experience

Jeferson F. Fonseca[A,F], *Maria Emilia F. Oliveira*[B], *Felipe Z. Brandão*[C], *Ribrio I. T. P. Batista*[C], *Alexandre R. Garcia*[D], *Pawel M. Bartlewski*[E] *and Joanna M. G. Souza-Fabjan*[C]

Theriogenology 86 (2016) 144–151

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

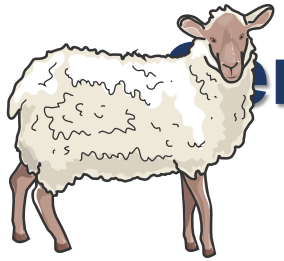
ELSEVIER

Review article

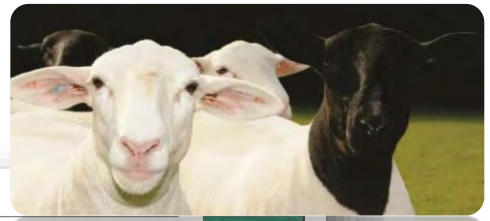
Nonsurgical embryo recovery and transfer in sheep and goats

Jeferson F. Fonseca [a,*], Joanna Maria G. Souza-Fabjan [b], Maria Emília F. Oliveira [c], Ceci R. Leite [b], Paula Maria P. Nascimento-Penido [d], Felipe Z. Brandão [b], Khoboso C. Lehloenya [e]

1 mg estradiol benzoate i.m. and 37.5 mg cloprostenol (Iv) 16 h before +
50 IU oxytocin i.v. 20 min prior to NSER



ervical dilation protocol - NSER in different breeds



Small Ruminant Research 220 (2023) 106914

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Different doses of pFSH are effective to promote follicular growth, superovulatory response, and embryo yield in White Dorper ewes

J.H. Dias [a], G.B. Vergani [b], J.D. Gonçalves [b], T.A. Oliveira [c], J.M. Penitente-Filho [a], V.S.A. Pereira [d], S.N. Esteves [d], A.R. Garcia [d], R.I.T.P. Batista [c], M.E.F. Oliveira [b], J.M.G. Souza-Fabjan [c], J.F. Fonseca [e,*]

BIOPRESERVATION AND BIOBANKING
Volume 00, Number 00, 2021
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DOI: 10.1089/bio.2021.0066

Nonsurgical Embryo Recovery as a Feasible Tool for Supporting Embryo Biobanks of Locally Adapted Brazilian Sheep and Goats

Jeferson F. Fonseca,[1,i] Gabriel B. Vergani,[2,ii] Monalisa S.D. Lima,[3] Kleibe M. Silva,[1] Alexandre W.U. Monteiro,[1] Alexandre F. Ramos,[4,iii] Bruna R.C. Alves,[5,iv] Joanna M.G. Souza-Fabjan,[5,v] Maria E.F. Oliveira,[1,2] and Ribrio I.T.P. Batista[5]

Theriogenology 145 (2020) 238–246

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Theriogenology

journal homepage: www.theriojournal.com

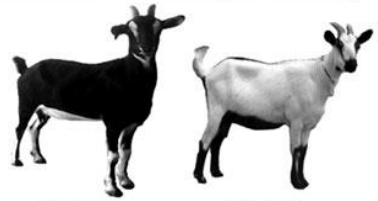
Preovulatory follicular dynamics, ovulatory response and embryo yield in Lacaune ewes subjected to synchronous estrus induction protocols and non-surgical embryo recovery

Lucas Machado Figueira [a, b], Nadja Gomes Alves [a, **], Joanna Maria Gonçalves Souza-Fabjan [b], Maria Emilia Franco Oliveira [c], Renato Ribeiro Lima [a], Guilherme Nunes Souza [b, d], Jeferson Ferreira Fonseca [e, *]

Brazilian naturalized sheep breeds

Morada Nova ($n = 20$) **Santa Inês** ($n = 20$) **Somalis** ($n = 20$)

Brazilian naturalized goat breeds

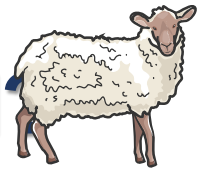


Canindé ($n = 15$) **Moxotó** ($n = 15$)

Estrus synchronization protocols

Superovulation

Cervical dilation protocol – is EB needed?



PhD student: Jenniffer Dias

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DOI: 10.1111/rda.13692

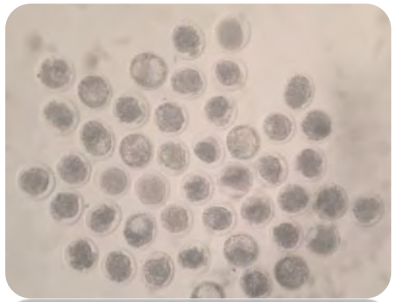
ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

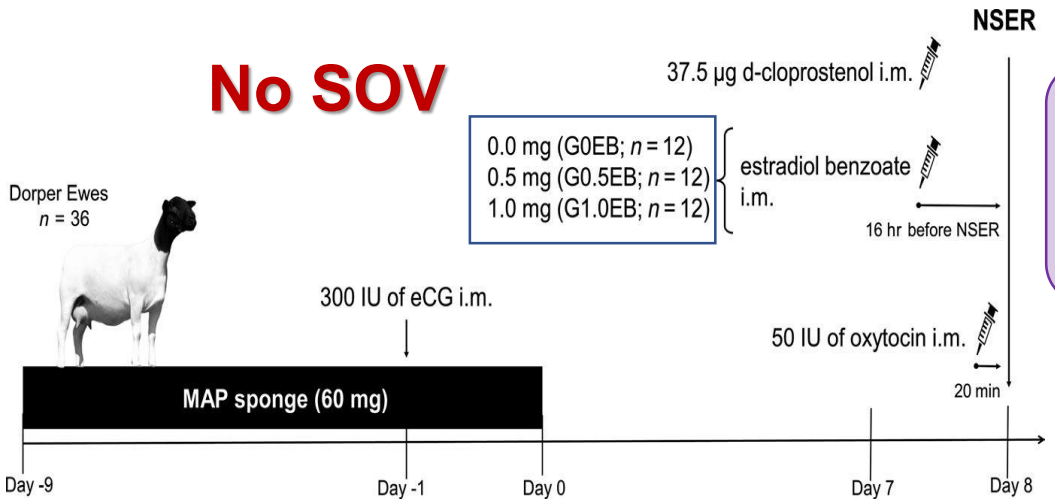
Successful transcervical uterine flushing can be performed without or reduced dose of oestradiol benzoate in cervical relaxation protocol in Dorper ewes

Jenniffer Hauschildt Dias[1] | Maria Amélia Pupin[2] | Gabriela Saloni Duarte[2] | Viviane Lopes Brair[3] | Cleber Jonas Carvalho de Paula[3] | Marco Antonio Paula de Sousa[4] | Ribrio Ivan Tavares Pereira Batista[3] | Joanna Maria Gonçalves Souza-Fabjan[3] | Maria Emília Franco Oliveira[2] | Jeferson Ferreira Fonseca[5]

EB ?



No SOV



EB may be either removed or reduced (dilation treatment) for **Dorper** ewes

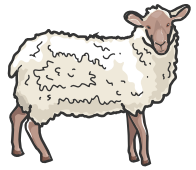
TABLE 1 Summary (mean ± *SEM* or %) of the cervical penetration attempts and embryo recovery procedures in pluriparous Dorper ewes who received different concentrations of oestradiol benzoate 16 hr before non-surgical embryo recovery (NSER) in a cervical relaxation induction protocol

	Oestradiol benzoate dose (mg)		
End Points	0.0	0.5	1.0
Number of animals	12	12	12
Mean number of corpora lutea/ewe*	2.0 ± 0.3 (19)	2.1 ± 0.3 (21)	1.7 ± 0.2 (20)
Hegar transposing successful (%)	100.0 (12/12)	91.7 (11/12)	100.0 (12/12)
Duration of Hegar dilator transposing (min)	4.2 ± 0.3^{a}	1.7 ± 0.3^{b}	1.6 ± 0.3^{b}
Number of cervical rings transposed	7.3 ± 0.3	7.4 ± 0.3	7.7 ± 0.4
Duration of mandrel/catheter transposing (min)	2.4 ± 0.5^{a}	1.6 ± 0.4^{ab}	1.3 ± 0.5^{b}
Duration of flushing (min)	19.2 ± 1.2	21.4 ± 1.4	18.6 ± 1.1
Total duration of procedure (min)	25.4 ± 1.6	24.0 ± 1.6	21.6 ± 1.2
NSER success (%)	83.3 (10/12)	91.7 (11/12)	100.0 (12/12)
Fluid recovery efficiency (%)	98.0 ± 2.0	99.0 ± 1.0	97.0 ± 1.6
Ewes with at least one structure recovered (%)*	70.0 (7/10)	60.0 (6/10)	58.3 (7/12)
Average structures recovered*	1.0 ± 0.3	0.9 ± 0.3	0.7 ± 0.2
Structure recovery (%)	52.6 (10/19)	39.1 (9/23)	40.0 (8/20)
Post-flushing fertility (%)	90.0 (9/10)^{A}	36.4 (4/11)^{B}	58.3 (7/12)^{AB}

a,b Means with different superscripts within rows differed (Tukey's test; $p < .05$).
A,B Means with different superscripts within rows differed (Fisher's exact test; $p < .05$).
( ) The values into the parenthesis indicate number of corpora lutea, animals or structures.
*Only ewes successfully flushed and with at least one CL counted.

Cervical dilation protocol – Does EB affect embryo viability?

PhD student: Jenniffer Dias



Theriogenology 218 (2024) 208–213

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journal homepage: www.theriojournal.com

ELSEVIER

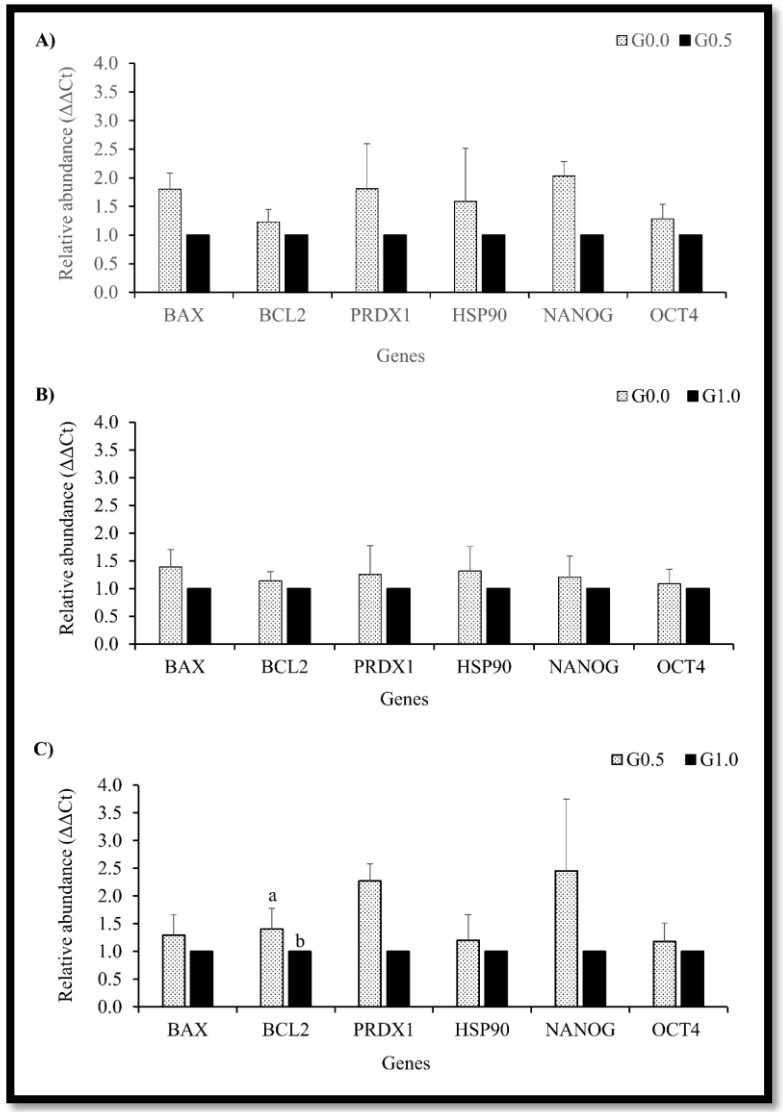
Original Research Article

The presence of estradiol benzoate in the cervical relaxation treatment for non-surgical embryo collection does not impair embryonic morphological quality, cryosurvival, and gene expression profile

Ribrio Ivan Tavares Pereira Batista[a,*], Jenniffer Hauschildt Dias[b], Joedson Dantas Gonçalves[c], Gabriel Brun Vergani[c], Maria Emilia Franco Oliveira[c], Joanna Maria Gonçalves Souza-Fabjan[a], Jeferson Ferreira Fonseca[d]

EB Group	n	Embryo *in vitro* survival (%)	
		24 h	48 h
G0.0	25	52 (13/25)	**60** (15/25)
G0.5	24	50 (12/24)	**54** (13/24)
G1.0	24	54(13/24)	**58** (14/24)

~ 57.5%



NSER technique

Cervical dilation

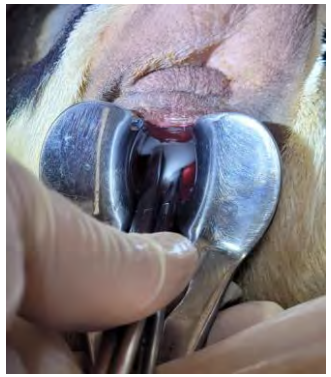
(EB + PGF + OT)

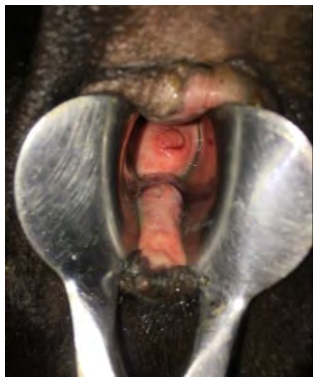
(PGF)

Sedation + Analgesia (hyoscine-N-butylbromide, sodium dipyrone and acepromazine maleate)

Restrained in an (elevated) cart

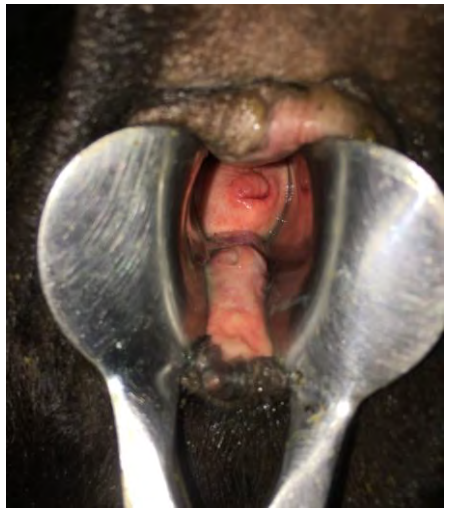
local anesthetics (epidural and pericervical gauze)



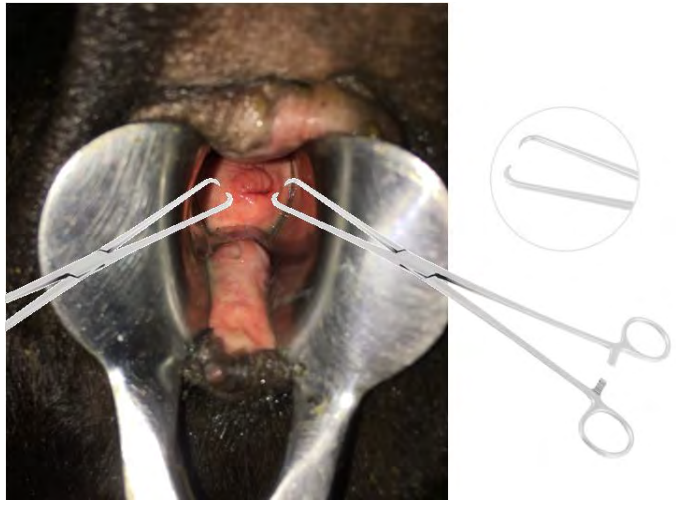


NSER technique

Collin speculum

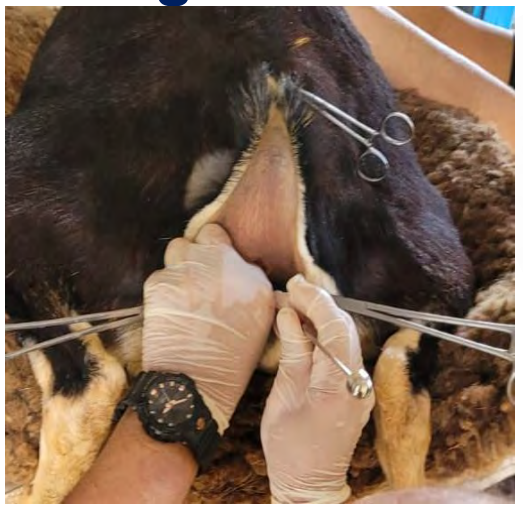


Pozzi forceps



Cervical traction

Hegar dilator



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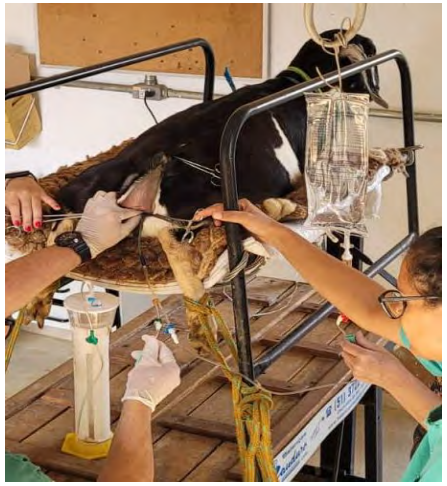
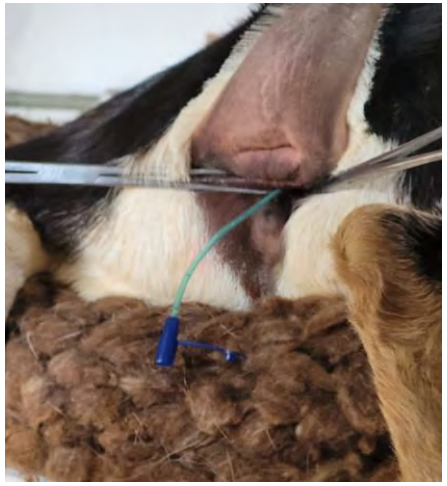
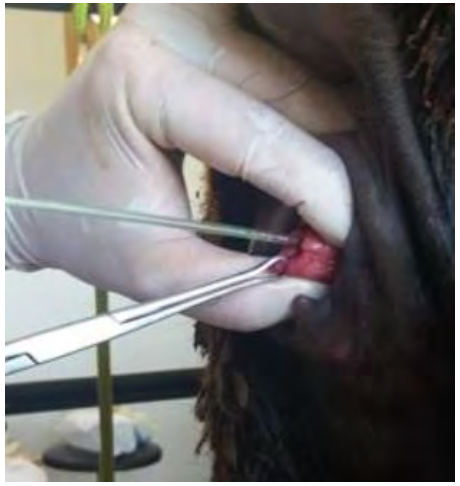
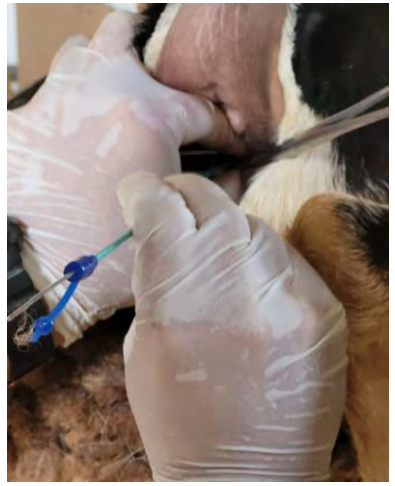


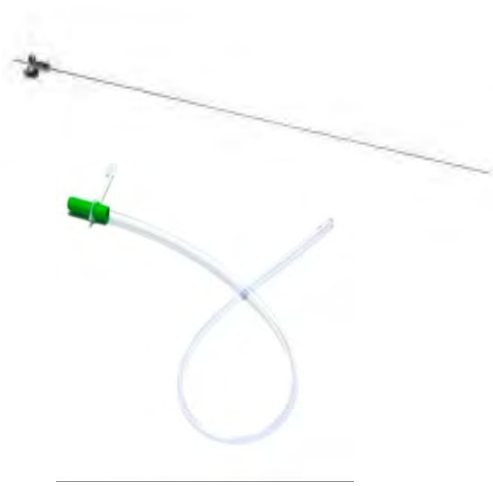
Mandrel removal



Catheter stiffened with a metal mandrel







Embryo collection techniques: efficiency of each one?

Laparotomy

NSER

BIOPRESERVATION AND BIOBANKING
Volume 20, Number 2, 2022
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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,[1] Ribrio I.T.P. Batista,[1] Luciana M. Melo,[2] Marcos A.L. Oliveira,[3] Maiana S. Chaves,[4] Jeferson F. Fonseca,[5] and Vicente J.F. Freitas[4]

Theriogenology 153 (2020) 112–121

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Transcervical *vs.* laparotomy embryo collection in ewes: The effectiveness and welfare implications of each technique

Juliana Dantas Rodrigues Santos [a,*], Rodolfo Ungerfeld [c], Mário Felipe Alvarez Balaro [a], Joanna Maria Gonçalves Souza-Fabjan [a], Isabel Oliveira Cosentino [a], Viviane Lopes Brair [a], Clara Vieira de Souza [a], Pedro Henrique Nicolau Pinto [a], Ana Luiza Cunha Bade [a], Jeferson Ferreira da Fonseca [b], Felipe Zandonadi Brandão [a,**]

Small Ruminant Research 158 (2018) 15–18

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Inflammatory markers in ewes submitted to surgical or transcervical embryo collection

F.C. Oliveira[a], C.S. Haas[a], C.E.R. Ferreira[a], K.L. Goularte[a], L.M.C. Pegoraro[c], B.G. Gasperin[a], A. Schneider[a], R.G. Mondadori[b], T. Lucia Jr.[a,*], A.D. Vieira[a]

Duration time
Fluid recovery %
Embryo recovery %
Nb of structures collected

NSER technique – welfare?

Theriogenology 153 (2020) 112–121

Contents lists available at ScienceDirect

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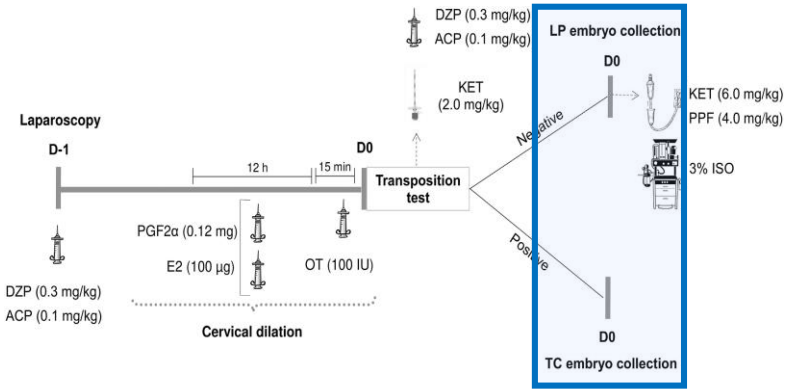
Transcervical *vs.* laparotomy embryo collection in ewes: The effectiveness and welfare implications of each technique

Juliana Dantas Rodrigues Santos [a,*], Rodolfo Ungerfeld [c], Mário Felipe Alvarez Balaro [a], Joanna Maria Gonçalves Souza-Fabjan [a], Isabel Oliveira Cosentino [a], Viviane Lopes Brair [a], Clara Vieira de Souza [a], Pedro Henrique Nicolau Pinto [a], Ana Luiza Cunha Bade [a], Jeferson Ferreira da Fonseca [b], Felipe Zandonadi Brandão [a,**]

PhD Student: Juliana Dantas

We assessed physiological, endocrine, biochemical, and behavioral parameters during and after the collection procedures



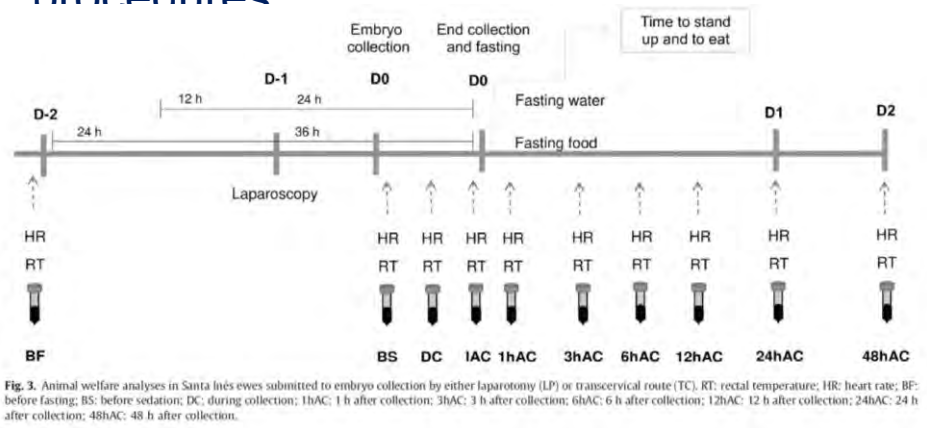


Fig. 3. Animal welfare analyses in Santa Inês ewes submitted to embryo collection by either laparotomy (LP) or transcervical route (TC). RT: rectal temperature; HR: heart rate; BF: before fasting; BS: before sedation; DC: during collection; 1hAC: 1 h after collection; 3hAC: 3 h after collection; 6hAC: 6 h after collection; 12hAC: 12 h after collection; 24hAC: 24 h after collection; 48hAC: 48 h after collection.

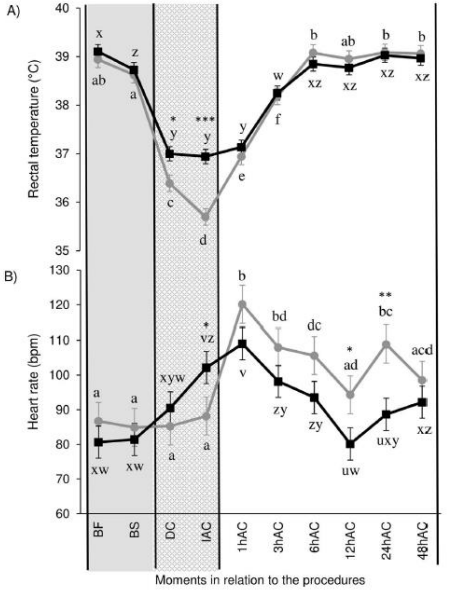


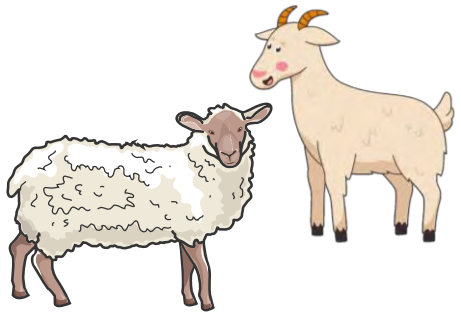
Table 2
Effect of embryo collection procedures, evaluation time, and their interactions on welfare parameters in Santa Inês sheep submitted to the cervical dilation hormonal protocol.

Parameter	Embryo collection procedures				*P*-value or significance		
	LP	SEM	TC	SEM	ECP	Time	ECPxTime
Rectal temperature (°C)	38.09	0.10	38.28	0.09	0.039	<0.0001	<0.0001
Heart rate (bpm)	98.04	2.69	91.64	2.48	0.010	<0.0001	0.037
Total protein (g/dL)	5.81	0.22	6.04	0.22	0.060	<0.0001	NS
Serum albumin (g/dL)	2.10	0.23	2.31	0.23	0.014	<0.0001	0.064
Serum globulin (g/dL)	3.72	0.07	3.74	0.06	NS	<0.0001	NS
Glycaemia (mg/dL)	70.51	3.34	72.74	3.06	NS	<0.0001	<0.0001
Cortisol (ng/mL)	32.34	2.69	29.52	2.23	NS	<0.0001	<0.0001

All data are presented as LSmeans. LP, laparotomy; TC, transcervical procedure; ECP, embryo collection procedure; ECPxTime, embryo collection procedure by time interaction; SEM, standard error; NS, non-significant.

most widespread

Embryo transfer techniques



Data on non-surgical embryo transfer (NSET) are scarce…

First report: Otsuki and Soma, 1964

Non-surgical embryo transfer (NSET)

Arq. Bras. Med. Vet. Zootec., v.66, n.2, p.613-616, 2014

Communication

[*Comunicação*]

Viable offspring after successful non-surgical embryo transfer in goats

[*Nascimento de fetos viáveis após transferência de embriões não-cirúrgica em caprinos*]

J.F. *Fonseca*[1], L.V. *Esteves*[2], F.N. *Zambrini*[3], F.Z. *Brandão*[2], M.G.C.D. *Peixoto*[4], R.S. *Verneque*[4], L.G.B. *Siqueira*[4], J.H.M. *Viana*[4]

Table 1. Records from recipient goats that received embryos by non-surgical transfer (via transcervical)

Parameter	Recipients				
	#8729	#8745	#8746	#8771	Total
Number of corpora lutea	3	2	1	2	8
Ovary / uterine horn	Left	Left	Left	Left	-
Embryos transferred	Mc-1*	Bl-1+Mc-3	Bl-1+Mc-3	Bl-1	6
Survival rate (%)	0.0 (0/1)	100.0 (2/2)	50.0 (1/2)	0.0 (0/1)	50.0 (3/6)

Ear-tag number of each recipient. * Embryo morphology and quality. ( ) Number of embryos.

Small Ruminant Research 192 (2020) 106215

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journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Factors affecting pregnancy rates for goat embryos recovered and transferred by transcervical route

Maria Clara C. Morais[a], Luciana V. Esteves[a], Joanna M.G. Souza-Fabjan[b], Maria Emília F. Oliveira[b,c], Marcio Roberto Silva[d], Felipe Z. Brandão[a], Jeferson F. Fonseca[a,*]

Table 1
Univariate and multivariate analyses for the pregnancy rates of recipient goats subjected to non-surgical embryo transfer.

Variable	Univariate model				Multivariate model	
	Total	Positive (%)	OR (CI 95 %)*	P-value	OR (CI 95 %)	P-value
Embryo stage						
Blastocyst	11	7 (63.6)	5.5 (1.2–25.6)	0.02	3.5 (0.6–19.3)	0.15
Morulae	25	6 (24.0)	1.0		1.0	
Embryo quality				0.05		0.27
1	14	8 (57.1)	11.97 (1.2–121.7)	0.03	7.24 (0.6–82.5)	0.11
2	12	4 (33.3)	4.48 (0.4–48.9)	0.21	4.30 (0.4–48.7)	0.23
3	10	1 (10.0)	1.00		1.00	

Multivariate model: Score test (p-value = 0.053); Hosmer and Lemeshow test (p-value = 0.34); Breslow-Day-Tarone test for homogeneity of OR (4.72; p-value = 0.094).

* OR: Odds ratio; CI: Confidence interval.

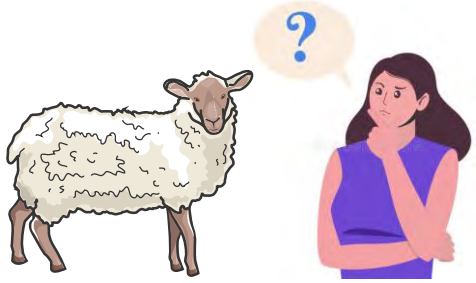
Candappa and Bartlewski *Reproductive Biology and Endocrinology* 2014, 12:8
http://www.rbej.com/content/12/1/8

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

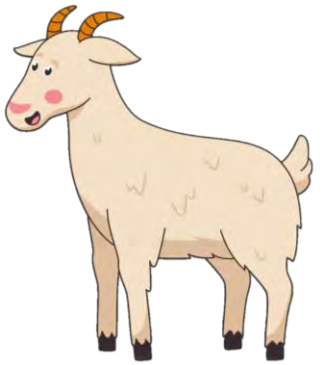
RESEARCH Open Access

Induction of cervical dilation for transcervical embryo transfer in ewes

Ivanka BR Candappa and Pawel M Bartlewski*

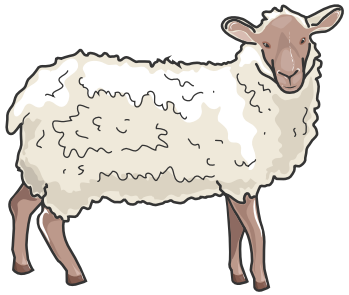


Overall pregnancy/kidding rate was 32%, representing an embryo survival rate of 36%



Final considerations

- ✓ Continued improvements are required to guarantee that the application of non-surgical AI/MOET is safe and maximizes the productivity outcomes in goats and sheep
- ✓ Regarding AI, its recent progress has been rather slow and the techniques used are generally like those developed in the last decades
- ✓ Considering the animal's well-being, the NSER method is a viable alternative to laparotomy and laparoscopy, which are more invasive but still predominantly used worldwide, possibly due to a lack of sufficient NSER training opportunities



NSER practical courses abroad

Costa Rica

Spain

Peru

Acknowledgements

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  - Dr. Felipe Zandonadi Brandão
  - Dr. Ribrio Ivan T. P. Batista
  - All colleagues and students
- **Embrapa Caprinos e Ovinos**
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  - Dr. Marie Saint-Dizier
- Universidad de Montevideo, Uruguay
  - Dr. Rodolfo Ungerfeld

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