https://doi.org/10.18406/2316-1817v15n120231753 e-ISSN 2316-1817

Timed artificial insemination in Serrana nanny-goats: the effects of vaginal specula and artificial insemination technicians

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Received in: 22/08/2022
Accepted in: 16/01/2023

Abstract

The success of artificial insemination (AI) depends on the equipment used, especially regarding the facilitation of artificial insemination technicians' work and the positioning of insemination guns in the genital tract of animals. This study aimed to evaluate how vaginal specula and artificial insemination technicians affected the fertility rates of timed artificially inseminated Serrana Transmontano goats (which are indigenous to Portugal). For this, 58 adult nanny-goats aged between three and nine years were used. They were reproductively controlled by a short progestogen treatment (FGA) (seven days) with equine chorionic gonadotropin (eCG). Timed cervical AI (43 hours after the end of the hormonal treatment) was performed with chilled semen. Nanny-goats were inseminated by two artificial insemination technicians (A *vs*. B) alternating two vaginal specula (Minitub *vs*. "Reyes"). Pregnancy was diagnosed by ultrasound 41 days later. About 98% of nanny-goats responded to our treatment with FGA and eCG. In total, 82.8% of nanny-goats were pregnant 41 days after AI. Neither vaginal specula (Minitub: 81.2% *vs*. "Reyes": 84.6%) nor artificial insemination technicians (A: 82.8% *vs*. B: 82.8%) affected fertility rates. Thus, we conclude that neither the vaginal specula used in this research nor the artificial insemination technicians affected Serrana goat pregnancy rates.

Keywords: Goat farming. Reproduction. Fertility rates.

Introduction

Multiple factors affect the fertility rates of small ruminants after artificial insemination (AI): genetics, individual characteristics, feeding, season, environment, reproductive activity control, semen collection and conservation, female management, cervix morphology, volume, insertion into the female genital tract, and semen deposition site (MORRELL, 2011; ARREBOLA et al, 2013).

AI success depends on its instruments since they can condition artificial insemination technicians' work; namely, location, fixation, and insertion of the gun in the cervical canal (QUINTAS et al, 2021; SILVA, 2022) and stress due to handling (PALACIOS, 2010; LEO, 2017). Cervical insemination causes higher fertility rates the deeper it deposits semen (CANDAPPA, BARTLEWSKI, 2011; VALENTINE et al, 2016). However, the possible trauma associated with attempting to overcome the second cervical fold decreases fertility rates as it causes a greater influx of immune cells to the lumen of the cervical canal, affecting the transport and viability of sperm in the female genital tract (CANDAPPA, BARTLEWSKI, 2011; KUMAR, NAQVI, 2014). Cervical trauma can also interrupt pregnancy in its early stages (KUMAR, NAQVI, 2014).

Artificial insemination technicians influences fertility rates in small ruminants (PALACÍN et al, 2012; FRANCIS, 2018). However, bibliography results differ, probably due to artificial insemination technicians' experience (PALACÍN et al, 2012; SILVA, 2022).

This study aimed to evaluate how vaginal specula and artificial insemination technicians affect the fertility rates of timed artificially inseminated Serrana Transmontano nanny-goats (which are indigenous to Portugal).

Material and methods

This study was conducted at the Instituto Politécnico de Bragança (ESA-IPB) Agrarian School in Quinta do Pinheiro Manso (Latitude 41° 48' 33''N, Longitude 6° 44' 3''W, and Altitude 670 meters), Bragança, Portugal, between April 1 and June 12, 2019.

Animals

In total, 58 Serrana Transmontano goats, aged between three and nine years, were used. Their last calving had occurred about six months before our experiment. Nanny-goats showed perfect conditions for reproduction and were randomly divided between treatments.

They grazed natural grassland and were supplemented in groups with hay from natural grasslands *ad libitum* and with 300 to 350 g/day of commercial concentrate. This energy/protein intake was maintained for 30 days after AI.

This assay first determined the body condition (BC) of the animals according to the classification table in Villaquiran et al. (2004), considering 0.25 intervals.

Reproductive control

On April 23, 2019, nanny-goats received a vaginal sponge impregnated with 20 mg of FGA (Chrono-Gest®, Intervet, Portugal). On the same date, they were administered an intramuscular injection with 100 μg pf Cloprostenol (Manure®, MSD Animal Health, Portugal). Progestogen treatment was maintained for seven days.

After removing the used vaginal sponges (April 30, 2019), the nanny-goats were administered an intramuscular injection with 300 IU of eCG (Intergonan®, Intervet, Portugal).

Response to reproductive control

To find the formation of the first corpus luteum after our FGA + eCG treatment, peripheral blood samples were collected within five days after eCG administration to determine plasma levels of progesterone (P4) via radioimmunoassay. For this, a DPC® Gamma C12 scintillation reader (Bertholt Technologies, Bad Wildbad, Germany) and *DiaSource* ® kits (ImmunoAssays, Louvain-la-Neuve, Belgium) were used. In this study, 7.1 and 13.3% mean coefficients of intra- and inter-assay variation were found, respectively.

First corpora lutea were considered formed when P4 plasma levels first exceeded 0.5 ng/ml.

Semen collection

Semen was collected with an electroejaculator (eProvac®, Minitub, model MT, Tiefenbach, Germany). Serrano goats had not ejaculated in three days.

After harvest, collecting tubes were transported to a laboratory and kept at 37°C in a refrigerated water bath (Neslab® RTE 221, Newington, USA). The Andromed® (Minitüb, Tiefenbach, Germany) which contained previously stored seminal thinners.

Seminal analyses

Semen volume was measured by the graduation in the collecting tubes. Sperm concentration and motility were estimated via Computer Assisted Sperm Analysis (CASA - Androvision®, Minitüb, Tiefenbach, Germany). The percentage of live sperm was determined via phase contrast trinocular microscopy (Motic BA-310, Barcelona, Spain) after semen drops were

diluted with two drops of eosin and a smear, prepared with 200 sperm.

Ejaculates showed a ≥ 2.0 mL volume, a ≥ 3.0 x 109 sperm mL-1 concentration, a ≥ 75% motility, and ≥ 75% of live sperm.

Seminal doses

After analyses, ejaculates were initially diluted (1:1) with Andromed;, corrected for volume by the values indicated by the Androvision program; their temperature, was decreased for about 90 minutes from 37°C to 15°C (Neslab® RTE 221, Newington, USA); and after ten minutes of rest, aspirated into 0.25-mL French straws, which were sealed with polyvinyl powder. The end of refrigeration and the beginning of AI were separated by about 30 minutes.

Each semen straw contained at least 350 x106 spermatozoa.

Timed artificial insemination

All nanny-goats were inseminated (May 2, 2019), regardless of whether they manifested estrus, 43 + 1 hours after eCG administration. AI were performed by two experienced artificial insemination technicians who alternately used two vaginal specula [Minitub® (Tiefenbach, Germany) and "Reyes" (Ovígén, Zamora, Spain)] (Figure 1) with LED light systems. Inseminations with the Minitub vaginal speculum were performed via Quicklock® guns for small ruminants (Minitube, Tiefenbach, Germany) and Minitub® cover sheaths (Tiefenbach, Germany), whereas those with the "Reyes" vaginal speculum, with IMV® universal guns for cattle (L'Aigle, France) and classical IMV® cover sheaths (L'Aigle, France).

Animals were inseminated in a breeding season in a stable. To facilitate the observation of the entrance of the equipment into the cervical canal of animals and for artificial insemination technicians' convenience, two team members raised the hind limbs of the chosen nanny-goats, maintaining their anterior limbs in contact with the ground.

Semen was always deposited as deeply as possible without forcing the passage of the insemination gun within the cervical canal.

Pregnancy diagnosis

A real-time Mindray Z5Vet ultrasound system with a 5.0-10.0 MHz multifrequency rectal probe was used 41 days after AI (June 12) to diagnose pregnancy in our sample.

Statistical analyses

Our experiment was completely randomized in a 2 x 2 factorial scheme with two vaginal speculum models and two artificial insemination technicians. An average of 15 goats were allocated for each speculum x artificial insemination technician combination. Variance analyses

Figure 1. Vaginal specula used in artificial insemination - Minitub (left) and "Reyes" (right).

were performed to find statistically significant differences between parameters (STEEL, TORRIE, 1980). The Bonferroni/Dunn test was used to compare means (DUNN, 1961) and the chi-squared test ($\chi 2$), frequencies (SNEDECOR, COCHRAN,1980).

Results and discussion

Our sample showed a mean age of 4.9 ± 1.6 years (cv = 31.5%). Zhang et al (2009) and Browning et al (2011) found lower fertility rates among nulliparous and primiparous nanny-goats (aged one to two years) than in those aged four to six years. Rhone et al (2013) found an increase in fertility rate between two and nine years of age.

The evaluated nanny-goats showed a 3.4 ± 0.4 BC (cv = 11.0%). The parameter can influence their fertility rates (ABSY et al, 2001 and KHANAL, 2016). According to Scaramuzzi and Martin (2008), Karikari and Blasu (2009), and Valentim et al (2015a), the best fertility rates emerge when goats show a 2.5-3.5 BC.

We found insignificant age differences ($P>0.05$) between nanny-goats inseminated by both vaginal specula (Minitub: 5.1 ± 1.4 years *vs*. " Reyes": 4.8 ± 1.7 years) and artificial insemination technicians (A:4.6 ± 1.4 years *vs*. B: 5.3 ± 1.6 years) as we did for their BC ($P>0.05$) for both vaginal specula (Minitub: 3.3 ± 0.4 points *vs*. " Reyes": 3.5 ± 0.3 years) and artificial insemination technicians (A:3.4 ± 0.3 points *vs*. B: 3.4 ± 0.5 points).

Response to the FGA + eCG treatment

Research has several hormonal methods to synchronize breeding periods – CIDR, vaginal sponges, subcutaneous progestogen auricular implants and two subcutaneous injections of F2α prostaglandins or its analogues (VALENTIM et al, 2015b; OMONTESE et al, 2016; HASHEMI, SAFDARIAN, 2017). The physiological response of females may vary according to device and hormone types (ROMANO, 2004, PADILHA et al, 2011 and SANTOS-NETO et al, 2015).

Progestogen-laden vaginal sponges can effectively synchronize the breeding period of caprine individuals (VALENTIM et al, 2015b; LEO, 2017; FRANCIS, 2018). Research may complement treatments to control ovarian activity with a single eCG injection (OMONTESE et al, 2016; LEO, 2017; FRANCISCO, 2018) to ensure adequate synchronization rates, guarantee satisfactory ovarian responses, and anticipate ovulation (RITAR et al, 1984; OMONTESE et al, 2016). We found that 98.3% (n = 57) of our sample responded to our hormonal treatment, proving its efficiency. Leão (2017) and Francisco (2018) observed similar results: 94.2% ($\chi 2=2.1$; $P>0.05$) and 98.2% ($\chi 2=0.0$), respectively.

Fertility rates after artificial insemination

We found that 82.8% (n = 40) of our sample were pregnant 41 days after AI. This result resembles that in Leo (2017) — 74.6% ($\chi 2=1.9$; $P>0.05$) — but exceeded that in Francisco (2018), 65.4% ($\chi 2=8.4$; $P\leq 0.01$). However, Francisco (2018) experienced some inconsistencies with the quantity and quality of the semen available for AI.

The new "Reyes" vaginal speculum, developed at Ovígén (Centro de selección y mejora genética del ganado Ovino y Caprino de Castilla y León, Spain), failed to improve fertility rates (Minitub: 81.2% *vs*. "Reyes": 84.6%; $\chi 2=0.6$; $P>0.05$). Silva (2022) found a similar result in Churra Galega Bragançana nanny-goats. However, it facilitated finding, fixing, and inserting our inseminating guns in the cervical canal of the evaluated animals and sped up handling. Quicker AI reduces stress due to handling females (PALACIOS, 2010; LEO, 2017), which tends to decrease fertility rates as it negatively affects the fertilization mechanism (SILVA et al, 2016; LEO, 2017).

Artificial insemination technicians can also influence fertility rates (STEYN, 2003; PALACÍN et al, 2012; FRANCISCO, 2018) since some individuals perform more efficiently than others (PALACÍN et al, 2012). Differences among these workers may result from variations in the time spent in each insemination (WINDSOR, 1995), their ability to navigate cervix folds (EPPLESTON, MAXWELL, 1993; SANTOLARIA et al, 2011; FRANCISCO, 2018) without forcing the passage of the guns used (SANTOLARIA et al, 2011; VALENTIM et al, 2016; FRANCISCO, 2018), the depth at which they deposit semen in the cervical canal (SALVADOR et al, 2005; CANDAPPA, BARTLEWSKI, 2011; FRANCISCO, 2018), the speed at which they deposit it (too fast would increase cervical reflux) (CANDAPPA, BARTLEWSKI, 2011; MORRELL, 2011), among others. In Francisco (2018), the inseminating team's experience conditioned fertility rates. In this study, artificial insemination technicians failed to influence fertility rates (A: 82.8% *vs*. B: 82.8%; $\chi 2=0.0$), as in Salvador et al. (2005). We may explain our results by artificial insemination technicians' large experience in the activity.

Conclusions

Vaginal specula and artificial insemination technicians failed to affect Serrana goat fertility rates after AI.

Acknowledgments

This study was conducted within 0687_OVISPID_2_E POCTEP - Interreg V-A Spain-Portugal.

References

ABSY, G.; ABUZEAD, S. M. M.; ZEIDAN, A. E. Resumption of postpartum ovarian activity in goats as affected by kidding season and body condition score under Egyptian conditions. Indian Journal of Animal Science, v. 71, n. 10, p. 922-926, 2001. Disponível em: https://epubs.icar.org.in/index.php/IJAnS/article/view/37053 Acesso em: 29 jun. 2022.

ARREBOLA, F.; GONZÁLEZ, O.; TORRES, R.; ABECIA, J. A. Artificial insemination in Payoya goats: Factors affecting fertility. Animal Production Science, v. 54, n. 3, p. 356-362, 2013. Disponível em: https://www.researchgate.net/publication/256382875_Artificial_insemination_in_Payoya_goats_Factors_affecting_fertility. Acesso em: 29 jun. 2022.

BROWNING, J. R. R.; BROWNING, M. L.; BYARS, M. Reproductive and health trait among Boer, Kiko, and Spanish meat goat does under humid, subtropical pasture conditions of the southeastern United States. Journal of Animal Science, v. 89, n. 3, p. 648-660, 2011. Disponível em: https://academic.oup.com/jas/article/89/3/648/4764236?login=true. Acesso em: 29 jun. 2022.

CANDAPPA, I. B. R.; BARTLEWSKI, P. M. A review of advances in artificial insemination (AI) and embryo transfer (ET) in sheep, with the special reference to hormonal induction of cervical dilation and its implications for controlled animal reproduction and surgical techniques. The Open Reproductive Science Journal, v. 3, p. 162-175, 2011. Disponível em: https://benthamopen.com/ABSTRACT/TORSJ-3-162. Acesso em: 29 jun. 2022.

DUNN, O. J. Multiple comparisons among means. Journal of the American Statistical Association, v. 56, p. 52-64, n. 293, 1961. Disponível em: https://sci2s.ugr.es/keel/pdf/algorithm/articulo/1961-Bonferroni_Dunn-JASA.pdf. Acesso em: 29 jun. 2022.

EPPLESTON, J.; MAXWELL, W. C. M. Recent attempts to improve the fertility of frozen ram semen inseminated into the cervix. Wool Sheep Breed, v. 41, n. 3, p. 291-302, 1993. Disponível em: https://sheepjournal.net/index.php/WTSB/article/view/1271. Acesso em: 29 jun. 2022.

FRANCISCO, L. F. Sincronização de cios e inseminação artificial em cabras das raças Serrana e Preta de Montesinho. Efeitos da suplementação multivitamínica, tratamento progestagénico curto + gonadotropina coriónica e método de preservação do sémen. 2018. 76 p. Dissertação (Mestrado). Escola Superior Agrária de Bragança, Bragança, Portugal.

HASHEMI M.; SAFDARIAN, M. Efficiency of different methods of estrus synchronization followed by fixed time artificial insemination in Persian downy does. Animal Reproduction, v. 14, n. 2, p. 413-417, 2017. Disponível em: https://www.animal-reproduction.org/article/doi/10.21451/1984-3143-AR825. Acesso em: 29 jun. 2022.

KARIKARI, P. K.; BLASU, E. Y. Influence of nutritional flushing prior to mating on the performance of West African Dwarf goats mated in the rainy season. Pakistan Journal of Nutrition, v. 8, n. 7, p. 1068-1073, 2009. Disponível em: https://docsdrive.com/pdfs/ansinet/pjn/2009/1068-1073.pdf. Acesso em: 29 jun. 2022.

KHANAL, P. Influence of crossbreeding and non genetic factors on doe fitness traits of Boer F1 and foundation breeds in southeastern United States. 2016. p. 82. Dissertação (Mestrado). Tenessee State University, Nashville, EUA.

KUMAR, D.; NAQVI, S. M. K. Effect of time and depth of insemination on fertility of Bharat Merino sheep inseminated trans-cervical with frozen-thawed semen. Journal of Animal Science and Technology, v. 56, n. 8, p. 1-6, 2014. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4540290/pdf/40781_2014_Article_7.pdf. Acesso em: 29 jun. 2022.

LEÃO, A. S. M. Ensaio da atividade reprodutiva e inseminação artificial de cabras de raça Serrana – ecótipo Transmontano – em Maio. Avaliação dos efeitos da dose eCG, do diluidor e do processo de preservação do sémen. 2017. p. 50. Dissertação (Mestrado). Escola Superior Agrária de Bragança, Bragança, Portugal.

MORRELL, J. M. Artificial insemination: current and future trends. In: MANAFI, M. Artificial insemination in farm animals. Rijeka: InTech, 2011, p. 1-14.

OMONTESE, B. O.; REKWOT, P. I.; ATE, I. U.; AYO, J. O.; KAWU, M. U.; RWUAAN, J. S.; NWANNENNA, A. I.; MUSTAPHA, R. A.; BELLO, A. A. An update on oestrus synchronisation of goats in Nigeria. Asian Pacific Journal of Reproduction, v. 5, n. 2, p. 96-101, 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S2305050016000117. Acesso em: 29 jun. 2022.

PADILHA, R. T.; MAGALHÃES, D. M.; MAIA-JUNIOR, A.; BRASIL, A. F.; ARAÚJO, A.A. Efeito de diferentes dispositivos intravaginais na sincronização estral e taxa de gestação em ovelhas deslanadas submetidas a IATF via cervical superficial com sêmen refrigerado. Revista Brasileira de Ciências Agrárias, v. 6, n. 3, p. 538-543, 2011. Disponível em: http://www.agraria.pro.br/ojs32/index.php/RBCA/article/view/v6i3a896/1071. Acesso em: 29 jun. 2022.

PALACÍN, I.; YÁNIZ, J. L.; FANTOVA, E.; BLASCO, M. E.; QUINTÍN-CASORRÁN, F. J.; SEVILLA-MUR, E.; SANTOLARIA, P. Factors affecting fertility after cervical insemination

with cooled semen in meat sheep. Animal Reproduction Science, v. 132, n. 3-4, p. 139-144, 2012. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0378432012001327. Acesso em: 29 jun. 2022.

PALACIOS, C. R. Manejo del semen e inseminación artificial. In: ABECIA, A. M.; FORCADA. F. Manejo reprodutivo en ganado ovino. Saragoça: Editora Servet, 2010. p. 111-144.

QUINTAS, H.; SILVA, L.; MATEUS, O.; RAMOS, J. J.; FERRER, L. M.; RUIZ, H.; LACASTRA, D.; VALENTIM, R. Synchronization of estrous using FGA internal pessaries and CIDR in Churra Galega Bragançana ewes. In: ECRHM COMMUNICATIONS, ISVA VIRTUAL MEETING, 2021.

RHONE, J. A.; WALDRON, D.F.; HERRING, A. D. Performance of Boer-Spanish and Spanish goats in Texas I: Body weights, fertility, prolificacy, and number of kids weaned. Journal of Animal Science, v. 91, n. 10, p. 4679-4683, 2013. Disponível em: https://academic.oup.com/jas/article-abstract/91/10/4679/4717165. Acesso em: 29 jun. 2022.

RITAR, A. J.; MAXWELL, W. M. C.; SALAMON, S. Ovulation and LH secretion in the goat after intravaginal progestogen sponge-PMSG treatment. Journal of Reproduction & Fertility, v. 72, n. 2, p. 559-563, 1984. Disponível em: https://pubmed.ncbi.nlm.nih.gov/6210363/. Acesso em: 29 jun. 2022.

ROMANO, J. E. Synchronization of estrus using CIDR, FGA or MAP intravaginal pessaries during the breeding season in Nubian goats. Small Ruminant Research, v. 55, n. 1-3, p. 15-19, 2004. Disponível em: https://www.sciencedirect.com/science/article/pii/S0921448804000069. Acesso em: 29 jun. 2022.

SALVADOR, I.; VIUDES-DE-CASTRO, M. P.; BERNACER, J.; GÓMEZ, E. A.; SILVESTRE, M. A. Factors affecting pregnancy rate in artificial insemination with frozen semen during non-breeding season in Murciano-Granadina goats: a field assay. Reproduction in Domestic Animals, v. 40, n. 6, p. 526-529, 2005. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/j.1439-0531.2005.00624.x. Acesso em: 29 jun. 2022.

SANTOLARIA, P.; PALACIN, I.; YANIZ, J. Management factors affecting fertility in sheep. In: MANAFI, M. Artificial insemination in farm animals. Rijeka: InTech, 2011. p 167-190.

SANTOS-NETO, P. C. DOS; GARCÍA-PINTOS, C.; PINCZAK, A.; MENCHACA, A. Fertility obtained with different progestogen intravaginal devices using short-term protocol for fixed-time artificial insemination (FTAI) in sheep. Livestock Science, v. 182, p. 125-128, 2015. Disponível em: https://www.sciencedirect.com/science/article/pii/S1871141315300317. Acesso em: 29 jun. 2022.

SCARAMUZZI, R. J.; MARTIN, G. B. The importance of interactions among nutrition, seasonality and socio-sexual factors in the development of hormone-free methods for controlling fertility. Reproduction in Domestic Animals, v 43, n. 2, p. 129-36, 2008. Disponível em: https://pubmed.ncbi.nlm.nih.gov/18638114/. Acesso em: 29 jun. 2022.

SILVA, L.T.V. Controlo reprodutivo e inseminação artificial com sémen fresco e refrigerado em ovelhas da raça Churra Galega Bragançana. Efeitos do dispositivo vaginal (esponjas ou CIDR), do vaginoscópio e do inseminador. 2022. 65 p. Dissertação (Mestrado). Escola Superior Agrária de Bragança, Bragança, Portugal.

SILVA, T. P. D.; TORREÃO, J. N. C.; MARQUES, C. A. T.; ARAÚJO, M. J.; BEZERRA, L. R.; DHANASEKARAN, K. D.; SEJIAN, V. Effect of multiple stress factors (thermal, nutritional and pregnancy type) on adaptive capability of native ewes under semi-arid environment. Journal of Thermal Biology, v. 59, p. 39-46. 2016. Disponível em: https: //www.sciencedirect.com/science/article/pii/S0306456516300365?via%3Dihub. Acesso em: 29 jun. 2022.

SNEDECOR, G. W.; COCHRAN, W. G. Statistical methods. 7.ed. Ames: Iowa State University Press, 1980. 185 p.

STEEL, R. G. D.; TORRIE, J. H. 1980. Principles and procedures of statistics. 2.ed. Nova Iorque: McGraw-Hill Company, 1980. 633 p.

STEYN, J. J. Application of artificial insemination (AI) on commercial sheep and goat production. In: SIMPÓSIO INTERNACIONAL SOBRE CAPRINOS E OVINOS DE CORTE, 2. Simpósio Internacional sobre Agronegócio da Caprinocultura Leiteira. João Pessoa, 2003. p. 367-379.

VALENTIM, R. C.; RODRIGUES, I.; MONTENEGRO, T.; SACOTO, S.; AZEVEDO, J., 2015a. Maneio reprodutivo em ovinos e caprinos. 4. Controlo da atividade reprodutiva em pequenos ruminantes – métodos naturais. Agrotec, v. 17, p. 19-23, 2015a. Disponível em: https://www.researchgate.net/publication/282327460_Maneio_reprodutivo_em_ovinos_e_caprinos_4_Controlo_da_atividade_reprodutiva_em_pequenos_ruminantes_-_metodos_naturais. Acesso em: 29 jun. 2022.

VALENTIM, R. C; RODRIGUES, I.; MONTENEGRO, T.; SACOTO, S.; AZEVEDO, J. Maneio reprodutivo em ovinos e caprinos. 5. Controlo da atividade reprodutiva em pequenos ruminantes – métodos hormonais. Agrotec, v. 18, p. 25-28, 2015b. Disponível em: https://www.researchgate.net/publication/289376854_Maneio_reprodutivo_em_ovinos_e_caprinos_5_Controlo_da_atividade_reprodutiva_em_pequenos_ruminantes_-_metodos_hormonais. Acesso em: 29 jun. 2022.

VALENTIM, R. C; RODRIGUES, I.; MONTENEGRO, T.; SACOTO, S.; AZEVEDO, J.; GOMES, M. J. Maneio reprodutivo em ovinos e caprinos. 7. Inseminação artificial em ovinos e caprinos. Agrotec, v. 21, p. 10-13, 2016. Disponível em: https://repositorio.utad.pt/bitstream/10348/7748/1/AG20%20-%20Zootecnia%20-%20Maneio%20reprodutivo%20em%20ovino.pdf. Acesso em: 29 jun. 2022.

VILLAQUIRAN, M.; GIPSON, T. A.; MERKEL, R. C.; GOETSCH, A. L.; SAHLU, T. Body condition scores in goats. Langston: AIGR-Langston University, 2004, 8 p. Disponível em: http://www.luresext.edu/sites/default/files/BCS_fact sheet.pdf. Acesso em: 29 jun 2022.

WINDSOR, D. P. Factors influencing the success of transcervical insemination in merino ewes. Theriogenology, v. 43, n. 6, p. 1009-1018, 1995. Disponível em: https://pubmed.ncbi.nlm.nih.gov/16727688/. Acesso em: 29 jun. 2022.

ZHANG, C. Y.; CHEN, S. L.; LI, X.; XU, D. Q.; ZHANG, Y.; YANG, L. G. Genetic and phenotypic parameter estimates for reproduction traits in the Boer dam. Livestock Science, v. 125, n. 1, p. 60-65, 2009. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1871141309001206. Acesso em: 29 jun. 2022.