Investigação

OVARIAN SUPERSTIMULATION TREATMENT FOR MULTIPLE OVULATION AND EMBRYO TRANSFER PROGRAMS IN SHEEP

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ABSTRACT

Superovulation in sheep aims to provide hormonal preparations to stimulate growth and ovulation of a higher number of preovulatory follicles. It is a decisive step in ewes multiple ovulation and embryo transfer, although still has results with high variability. Several studies have been performed to obtain a homogeneous and satisfactory answer to this biotechnology, although a better understanding of this phenomena is still required, taking into account the estrous cycle, physiological hormonal regulation and biological phenomenon of follicular dynamics. Considering the importance of superovulation in MOET technique in sheep, this review aims to approach the key aspects of this event in ewes (reproductive physiological phenomena and treatment of superovulation).

KEY-WORDS: gonadotropin, ovine, reproductive biotechnology, superovulation.

INTRODUCTION

The sheep industry has been standing out as a profitable alternative production for producers (PACHECO and QUIRINO, 2010). This scenario results in the constant search for animals with high reproductive performance, therefore, it is necessary to develop and properly implement the reproductive biotechnologies in sheep. Among these, highlighting artificial insemination, estrus and ovulation synchronization and induction, embryo transfer, in vitro embryo production, early pregnancy diagnosis, embryo sexing, embryo cloning and transgenesis (SIMPLÍCIO et al. 2007).

In small ruminants, the multiple ovulation and embryo transfer (MOET) is a unique reality. The MOET primarily aims to produce a larger number of offspring in a shorter time, with remarkable quality to the herd, applying techniques that enable higher productivity of follicles, ovulation and embryos, resulting in embryo transfer and higher birth rate in sheep herd (OLIVEIRA et al. 2013c).

The use of ovarian superovulation techniques becomes essential for MOET to reach considerable results in researches and sheep breeding. In this context, studies have been performed to find the most suitable formulations of superstimulation protocols, which can be cost-effective with satisfactory and homogeneous results. Considering the importance of superovulation in MOET, this review aims to approach the major aspects of this event in sheep (reproductive physiological phenomena and treatment of superovulation).

ESTROUS CYCLE

The estrous cycle can be defined as a sequence of physiological events consisting of biochemical, morphological and physiological changes (ovaries and genital tract) which results in estrus manifestation, preparation of the genital tract of animals for ovulation, copulation, fertilization and embryo implantation (OLIVEIRA et al. 2013a).

The goats and sheep are seasonally polyestrous, short-day breeders, exhibiting estrus in a defined period of time during the year, where the number of hours of light per day is lower (photoperiod). This duration is defined primarily by latitude and secondarily by breed. Furthermore, as farther away from the equator line, the reproductive seasonality tends to be more evident. Furthermore, at lower latitudes the reproductive seasonality tends to decrease or even stop (continuously polyestrous) and nutritional conditions take place as the biggest influential factor of it (FONSECA et al. 2007). Regarding the breed factor, ewes that present reproductive activity throughout the year, even in areas close to the tropics (FONSECA, 2006).

In sheep, the average estrus cycle length is 17 days (MENCHACA et al. 2010), divided into follicular phase (four days), and luteal phase (13 days) (RUBIANES, 2000). The follicular phase is characterized by the estrous behavior, luteinizing hormone (LH) preovulatory peak and the onset of ovulation. The luteal phase is characterized by the presence of one or more corpus luteum (PINEDA, 1989), ending in luteolysis (MACMILLAN and BURKE, 1996). According to Thompson (2006), the estrous cycle can be divided into four phases: 1- estrus, the period of time when the female is sexually receptive, with ovulation usually at the end of this phase; 2- metaestrus, phase when occurs the initial development of the corpus luteum (luteogenesis); 3- diestrus, period when there is activity of a corpus

luteum, ending with this regression (luteolysis); and 4- proestrus, which begins shortly after the regression of the corpus luteum from the previous cycle and is characterized by follicular growth.

The central nervous system under action of gonadotropin releasing hormone (GnRH), stimulates the anterior pituitary to synthesize and secrete the hormones LH and follicle stimulating hormone (FSH) (MORELLO and CHEMINEAU, 2008). These hormones control follicular development and steroidogenesis, resulting in secretion of estrogen by granulosa cells from ovarian follicles and adrenal cortex (RUBIANES, 2000). With the elevation of plasma estrogen levels, occurs the characteristic estrous sexual behavior and progressive increase in LH pulse (CUMMING et al. 1971). The pre-ovulatory LH peak leads to the ovulation of preovulatory follicles followed by luteinization of the remaining follicular structure leading to subsequent formation of the corpus luteum (MENCHACA and RUBIANES, 2004).

The corpus luteum is responsible for progesterone production and secretion. Moreover, elevated serum levels of this hormone maintain low frequency of LH secretion (due to negative feedback to the hypothalamus, inhibiting GnRH), so the dominant follicles will not ovulate (GOODMAN and INSKEEP, 2006). At the end of the luteal phase, prostaglandine F2 alpha (PGF2a) is secreted by the endometrial glands of non-gravid uterus, inducing regression of the corpus luteum (OLIVEIRA et al. 2013a). However, when there is the fetus formation, there is secretion of interferon-tau, leading to maternal pregnancy recognition and maintenance of the corpus luteum (NISWENDER et al. 2000).

FOLLICULAR DYNAMICS

Follicular dynamics is defined as a continuous process of follicular growth and regression (BORGES et al. 2004). Follicles develop in a wave-like pattern during the interovulatory period (EVANS et al. 2000). Typically, there are three or four follicular waves present during this period (EVANS et al. 2003) at intervals of five days (RUBIANES, 2000).

At follicular wave, three events may be physiologically and morphologically characterized: recruitment, selection and dominance (GINTHER et al. 1996). During emergency of each wave occurs the recruitment of a group of follicles (approximately 2 mm) to continue their development. From those recruited follicles, only one to four are selected to complete their development and others suffer regression. The follicles that have reached a diameter of approximately 4 mm are selected to enter in dominant phase. These follicles have dominance over others and continue their development, and can reach the ovulatory size (≥ 5 mm) before initiating the process of regression or ovulation (MENCHACA et al. 2016).

In ewes, follicular growth can be subject to co-dominance because more than one follicle can achieve an ovulatory size in a single wave. Follicles originated from two consecutive waves can ovulate simultaneously (GONZALES-BULNES et al. 2005).

FOLLICULAR DYNAMICS ULTRASONOGRAPHY

The first studies were carried out by exploratory laparotomy, laparoscopy or ovaries obtained from slaughterhouses (BRAND and JONG, 1973; DRIANCOURT et al. 1985; NOEL et al. 1993). The application of transrectal ultrasonography started during the mid-90s (GINTHER

and KOT, 1994; RAVINDRA et al. 1994; RUBIANES et al. 1996). This imaging method is considered non-invasive, produces minimal stress or discomfort to the animal (WURST et al. 2007) and has been considered an important tool to evaluate follicular growth dynamics, ovarian response in estrus synchronization protocols (Barret et al. 2002) and superovulatory response in sheep (BARRET et al. 2012; MEINECKE-TILLMANN 2017).

In small ruminants, there are limiting aspects to study follicular dynamics which need to be considered, such as the ovaries size, their structures and the natural mobility of these gonads in the animals abdominal cavity, which requires professional adequate training before implementing the ultrasonographic examination (MEINECKE-TILLMANN 2017).

The ultrasonography enables monitoring throughout the ovulatory follicular dynamics: emergence, growth, maximum and ovulatory diameter and time of ovulation. There are few studies that approach these issues, and research in this area have great importance, particularly for the ovulatory time, because it allows to determine the exact moment to perform artificial insemination, considering the viable time of the oocyte and sperm.

In obstetrics and animal reproduction, the Doppler technique has been applied for evaluation and characterization of vascular blood flow in the ovarian tissue, uterus, fetus or placenta (PETRIDIS et al. 2017). In women, this method is used to monitor the response of the reproductive organs to hormonal treatments during in vitro fertilization protocols (ZAIDI et al. 1996). In veterinary medicine, studies have shown that Doppler technique helps to predict ovarian response after hormonal superstimulation protocols in cows (MATSUI and MIYAMOTO, 2009) and horses (WITT et al. 2012).

Specifically in superstimulated sheep, it has not been observed a positive correlation between hormone treatment and the follicles

blood flow (OLIVEIRA et al. 2014). These authors observed that the animals responded differently to superstimulation protocols, some sheep had greater blood flow than others, regardless of their experimental group, and there was no vascularization pattern according to the treatment used. It is suggested further studies as above, to establish the applicability of the Doppler technique in follicular assessment to superovulation treatments in sheep, in order to evaluate the relationship of follicular vascularization and ovarian and the animals embryonic response.

SUPEROVULATION IN EWES

Programs of multiple ovulation and embryo transfer have become popular and increasingly accessible to the producers (FONSECA et al. 2007). The benefits are the main reasons that motivate its spread and among its advantages, there is the ability to promote greater selection intensity, reduced generation interval (FONSECA et al. 2010) and achieving greater number of progeny of genetically superior females (GUSMÃO and ANDRADE MOURA, 2005). In addition, it provides technical basis for implementation of other related biotechnologies (REICHENBACH et al. 2001) facilitates trade procedures (nationally or internationally) of genetic material ensuring health (GUSMÃO and ANDRADE MOURA, 2005) and preservation of endangered species (BRASIL, 2013). The main disadvantage of MOET is the cost related to the drugs used, surgical technique and cryopreservation (BALARO and BRANDÃO, 2013).

Superovulation is one of the major steps of the MOET and has as principle the provision of hormones that stimulate the growth of a greater number of follicles and further ovulation (CLEMENT, 2013). However, a limiting factor in the use of MOET is the wide variation of superovulatory response (COGNIE, 1999; BALDASSARE, 2008). González-Bulnes et al. (2000) observed that this is a problem in about 20 to 30% of treated females.

It is believed that this variability in superovulation response is due to intrinsic and extrinsic factors related to animals, such as breed (GONZÁLEZ-BULNES et al. 2004), genetic, season, nutrition (GUSMÃO et al. 2013), method of mating (insemination) (BARI et al. 2000) and especially the superovulation protocol employed. The latter has been subject of intense research, and listed some potential attributes with detrimental effect to the responses (MACIEL, 2016).

For the basic protocols (synchronization) highlights the use of treatments which primarily consider the estrous cycle length, and not the biological phenomenon of animals follicular dynamics (MACIEL, 2016). In this sense, the progesterone profile induced by the devices used in the treatment (long term protocols) (OLIVEIRA et al. 2013a), follicular condition at the beginning of superstimulatory protocol (GONZÁLEZ-BULNES et al. 2002) and disability or absence of pre-ovulatory LH peak (luteinizing hormone) after treatment with gonadotropin (GONZÁLEZ-BULNES et al. 2003) are considered potential responsible for the variability results of these protocols. The gonadotropin treatment used in superstimulation has also been linked to heterogeneity of results (QUAN et al. 2011).

Exogenous gonadotropins used in superstimulation are equine chorionic gonadotropin (eCG), FSH and human menopausal gonadotropin (hMG) (FONSECA et al. 2007). The latter, although resulting in ovulation number similar to that obtained by eCG is not widely used because causes premature luteal regression (SCHIEWE et al. 1990).

The eCG was widely used in embryo transfer programs in sheep (ARMSTRONG and EVANS, 1983), however, due to its long half-life (72 hours), the use of high doses

of eCG for the animal superovulation induction and the time of application can cause persistent anovulatory follicles (AZAWI and AL-MOLA, 2011). The production of high amounts of estradiol by these anovulatory follicles can negatively impact the uterine environment, the uptake of oocytes by fimbriae and transport of sperm in the female reproductive tract (CHAGAS SILVA et al. 2003), affecting the number and viability of embryos. Moreover, antibodies formation is common after repeated treatments with eCG, resulting in lower efficiency because of inhibitory effect of stimulatory activity of the gonadotropin (ROY et al. 1999) and can affect the donor's fertility (LOPEZ-SEBASTIAN et al. 2006).

To improve the superovulation programs efficiency, it has been observed in the last three decades the replacement of eCG by porcine and ovine FSH (OLIVEIRA et al. 2013a). The use of FSH allows a higher ratio of female to produce viable embryos per superovulated sheep when compared to females treated with eCG (100% versus 55%, respectively) (ARMSTRONG and EVANS, 1983). In addition, the FSH is related to higher ovulation and fertility rates, producing better quality embryos, lower incidence of anovulatory follicles and more uniform individual response towards the eCG (ARMSTRONG and EVANS, 1983). It should be noted that the association of eCG with FSH at the beginning of superovulation treatment may be applied and contributes to the maintenance of high endogenous FSH concentrations during the gonadotropin treatment (BARTLEWSKI et al. 2009).

Usually, the superovulation treatment with FSH starts 48 hours before removal of the progestogen used for synchronization, for 2-4 days, with decreasing (COGNIE, 1999) and multiple (6 to 8 applications) consecutive doses (OLIVEIRA et al. 2013a). To properly stimulate follicles is necessary administering FSH at intervals of 12 hours, because their concentrations in the peripheral circulation decreases to basal levels after 10 hours (DEMOUSTIER et al. 1988), making it necessary to repeat administrations at a short time

intervals, which intensify management, making it less practical (MACIEL, 2016).

FSH doses used in farm animals superstimulation protocols vary depending on the species, body weight, breed and season (FONSECA, 2005), having great importance in obtaining homogeneous superovulatory responses (WU et al. 2011). In Brazil, protocols with total doses of 256 mg (Folltropin®) are commonly employed in sheep (OLIVEIRA et al. 2013a). Total doses of 175-200 mg has been employed with consistent performance (ovulation rate and production of viable embryos) when compared with higher doses (OLIVEIRA et al. 2013a; OLIVEIRA et al. 2014).

Although widely used, this exogenous FSH doses can be considered very high (FONSECA et al. 2007). Detailed studies characterizing efficient and smaller doses may increase the efficiency and decrease the variability of superovulation (FONSECA, 2005). Furthermore, the use of high doses in superovulation protocols may cause changes in ovarian and/or endocrine dynamics (KAFLI and MCGOWAN, 1997), on LH pulses (BEVERS et al. 1989), and the occurrence of early luteal regression (RODRIGUEZ, 2016). High doses can induce a prolonged stimulation on anovulatory follicles, which remain producing estrogen, leading to the persistence of high concentrations of this hormone during the early luteal phase, occurring early release of PGF2 α and luteal regression (FONSECA and SIMPLÍCIO, 2008). This phenomenon mainly results in a decreasing recovery rate and lower quality embryos (FONSECA et al. 2007). It is also worth mentioning that high doses have high costs and are not compatible with animal production (LOIOLA FILHO, 2013).

Few studies related to the use of lower FSH doses in sheep are more recent. Gibbons et al. (2010) tested two doses of FSH (200 mg and 80 mg) and observed that the recovered and transferable embryo number was similar between treatments, however with a lower ovulation rate for the lowest dose (80 mg). Furthermore,

Wu et al. (2011) compared the superovulation using 150 mg (6 applications) and 120 mg (3 applications) (Folltropin-V) not being observed differences in the numbers of recovered and transferable embryos, but it is noteworthy that in that study design cannot be established the actual effect related to response (dose or number of applications). In another study, the evaluation of total doses of 200 mg (6 applications) and 128 mg (6 applications) was performed, and there was no influence of dose reduction in the production of transferable embryos (LOIOLA FILHO et al. 2015). Moreover, Menezes et al. (2014) tested three doses of FSH (200 mg, 133 mg and 80 mg), noting that the average number of collected structures, viable and freezable embryos were similar among treatments, although superovulatory response was greater at the dosage of 200 mg and 133 mg when compared to 80 mg. Given these facts listed above, it is evident the need for further studies that help to achieve a better match of FSH doses for superovulation in ewes.

The results variability in ewes superovulation is a great challenge to be overcome by researchers. Further studies of the phenomena occurring in superovulation treatments are very important for satisfactory and homogeneous answers. Achieving these results a further expansion of multiple ovulation and embryo transfer technique in sheep will be possible, allowing a technological breakthrough in animal breeding companies and sheep producers.

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REFERENCES

Armstrong DT, Evans G. 1983. Factors affecting success of embryo transfer in sheep and goats. *Theriogenology*. 19(1):31-42.

Azawi OI, Al-Mola MKMA. 2011. A study on the effect of GnRH administration on the ovarian response and laparoscopic intrauterine insemination of Awassi ewes treated with eCG to induce superovulation. *Tropical Animal Health and Production*. 43(7):1351–1355.

Doc. Eletrônico (internet): Balaro M, Brandão FZ. MilkPoint. 2013. Regressão Prematura de Corpos Lúteos - RPCL na Múltipla Ovulação e Transferência de Embriões - MOTE em pequenos ruminantes. 1 p. Disponível em: <http://www.milkpoint.com.br> [Acessado em 10/2016].

Baldassare H. 2008. *Coleta, conservação e transferência de embrião*. In: Aisen EG. Reprodução ovina e Caprina. 1. ed. São Paulo: MedVet. pp. 143-152.

Bari F, Khalid M, Haresign W. et al. 2000. Effect of mating system, flushing procedure, progesterone dose, and donor ewe age on the yield and quality of embryos within a MOET program in sheep. *Theriogenology*. 53(3):727–742.

Barret DMW, Bartlewski PM, Cook SJ. et al. 2002. Ultrasound and endocrine evaluation of the ovarian response to PGF2α given at different stages of the luteal phase in ewes. *Theriogenology*. 58(7): 1409-1424.

Bartlewski PM, Fuerst KJ, Alexander BD. et. al. 2009. Systemic Concentrations of Endogenous and Exogenous FSH in Anoestrous Ewes Superstimulated with Follitropin-V. *Reproduction in Domestic Animal*, 44(2):353–358.

Bevers MM, Dieleman SJ, van Tol HTM. et al. 1989. Changes in pulsatile secretion patterns of LH, FSH, progesterone, androstenedione and oestradiol in cows after superovulation with PMSG. *Journal of Reproduction and Fertility*. 87(2):745–754.

Borges AM, Torres CAA, Rocha Júnior VR. et al. 2004. Dinâmica folicular e momento da ovulação em vacas não lactantes das raças Gir e Nelore durante duas estações do ano. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 56(3):346-354.

Brand A, Jong WHR. 1973. Qualitative and quantitative micromorphological investigation of tertiary follicle population during the oestrous cycle in sheep. *Journal of Reproduction and Fertility*. 33:431-439.

Brasil O.O. 2013. Maior tempo de exposição à progesterona associado ao uso de agonista de GnRH na superovulação e produção de embriões ovinos. 53f. Brasília, DF. Dissertação (Mestrado em Ciências Animais) – Programa de Pós-graduação em Ciências Animais, Universidade de Brasília.

Chagas e Silva J, Lopes Da Costa L, Cidadão R. et al. 2003. Plasma progesterone profiles, ovulation rate, donor embryo yield and recipient embryo survival in native Saloia sheep in the fall and spring breeding seasons. *Theriogenology*. 60(3):521–532.

Clemente CAA. 2013. Ativação e maturação folicular e produção embrionária em ovelhas da raça Santa Inês em diferentes condições climáticas. 100f. Belo Horizonte, MG. Tese (Doutorado em Ciência Animal) – Programa de Pós-graduação em Ciência Animal, Universidade Federal de Minas Gerais.

Cognie Y. 1999. State of Art in sheep-goat embryo transfer. *Theriogenology*. 51(1):105-116.

Cumming IA, Brown JM, Blockey MA. et al. 1971. Regulation of oestrous cycle in the ewe. *Journal of Reproduction and Fertility*. 24(1):148-149.

Demoustier MM, Beckers JF, Van Der Zwalm P. et al. 1988. Determination of porcine plasma levels during superovulation treatment in cows. *Theriogenology*. 30(2):379-386.

Driancourt MA, Gibson WR, Cahill LP. 1985. Follicular dynamics throughout the oestrous cycle in sheep. A review. *Reproduction Nutrition Development*. 25:1-15.

Driancourt MA, Webb R, Fry RC. 1991. Does follicular dominance occur in ewes? *Journal of Reproduction and Fertility*. 93(1):63-70.

Evans AC, Duffy P, Hynes N. et al. 2000. Waves of follicle development during the estrous cycle in sheep. *Theriogenology*. 53(3):699-715.

Evans AC. 2003. Ovarian follicle growth and consequences for fertility in sheep. *Animal Reproduction Science*. 78(3-4):289–306.

Doc. eletrônico (CD): Fonseca JF, Simplicio AA. 2008. Inseminação artificial e transferência de embriões em ovinos e caprinos. In: *Anais do Encontro Internacional da Pecuária da Amazônia – AMAZONPEC* (Belém, Brasil). 1 CD-ROM.

Fonseca JF, Souza JMG, Bruschi JH. 2007. Sincronização de estro e superovulação em ovinos e caprinos. In. *Simpósio de caprinos e ovinos da EV-UFGM*. Belo Horizonte, Brasil, p. 167-194.

Fonseca JF, Souza JMG, Camargo LSA. 2010. Estado da arte de ovócitos e embriões de caprinos e ovinos: passado, presente, futuro. *Acta Scientiae Veterinariae*. 38:353-369.

Fonseca JF. 2006. Otimização da eficiência reprodutiva em caprinos e ovinos. In. *Encontro Nacional de Produção de Caprinos e Ovinos*. Campina Grande, Brasil, p. 1-10.

Doc. eletrônico (CD): Fonseca JF. 2005. Estratégias para o controle do ciclo estral e superovulação em ovinos e caprinos. In: *Congresso Brasileiro de Reprodução Animal*. (Goiânia, Brasil). 1 CD-ROM.

Gibbons A, Pereyra-Bonnet F, Escobar L. et al. 2010. Eficiencia de un tratamiento de ovulación múltiple con dosis reducida de FSHp en ovejas Merino. In. *Segunda Jornada Internacionales del Instituto de Investigación y Tecnología en Reproducción Animal* (INITRA). Buenos Aires, Argentina, p. 268.

Ginther OJ, Kot K. 1994. Follicular dynamics during the ovulatory season in goats. *Theriogenology*. 42(6):987-1001.

Ginther OJ, Wiltbank MC, Fricke PM, et al. 1996. Selection of the dominant follicle in cattle. *Biology of Reproduction*. 55(6):1187-1194.

González-Bulnes A, Berlinquer F, Cocero MJ. et al. 2005. Induction of the presence of corpus luteum during superovulatory treatments enhances in vivo and in vitro blastocysts output in sheep. *Theriogenology*. 64(6):1392-1403.

González-Bulnes A, Santiago-Moreno J, Gomez-Brunet A. et al. 2000. Relationship between ultrasonographic assessment of the corpus luteum and plasma progesterone concentration during the oestrous cycle in monovular ewes. *Reproduction in Domestic Animals*. 35(2):65-68.

González-Bulnes A, Baird DT, Campbell BK. et al. 2004. Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reproduction Fertility and Development*. 16(4):421–435.

González-Bulnes A, Carrizosa JA, Díaz-Delfa C. et al. 2003. Effects of ovarian follicular status on superovulatory response of dairy goats to FSH treatment. *Small Ruminant Research*. 48(1):9-14.

González-Bulnes A, Santiago-Moreno J, Cocero MJ. et al. 2002. Measurement of inhibin A predicts the superovulatory response to exogenous FSH in sheep. *Theriogenology*. 57(4): 1263-1272.

Goodman RL, Inskeep EK. 2006. *Neuroendocrine control of the ovarian cycle of the sheep*. In: Neill JD. Knobil and Neill's: Physiology of Reproduction. 3. ed. Amsterdam: Elsevier. pp. 2389-2447.

Gusmão AL, Andrade Moura JC. 2005. Transferência de embriões em caprinos e ovinos. *Acta Scientiae Veterinariae*. 33:29-33.

Gusmão AL, Biscarde CEA, Kiya CK. 2013. Superovulação e transferência de embriões em ovelhas. *Revista Brasileira de Reprodução Animal*. 37(2):226-231.

Kafi M, McGowan MR. 1997. Factors associated with variation in the superovulatory response of cattle. *Animal Reproduction Science*. 48(2-4):137–157.

Loiola Filho JB, Monte APO, Souza TTS. et al. 2015. Effect of pFSH dose reduction on in vivo embryo production in Dorper ewes. Semina: *Ciências Agrárias*. 36(6):4215-4224.

Loiola Filho JB. 2013. Efeito da dose de pFSH na produção *in vivo* de embriões em ovelhas dorper pertencentes a duas categorias de peso corporal. 65f. Petrolina, PE. Dissertação (Mestrado em Ciência Animal) – Curso de Pós-graduação em Ciência Animal, Universidade Federal do Vale do São Francisco.

López-Sebastián A, Gonzáles-Bulnes A, Moreno JS. 2006. Control y manejo reproductivo en pequeños rumiantes. In: *XXIX Curso Internacional de Reproducción animal*. Madri, Espanha, p. 43-52.

Macmillan KL, Burke CR. 1996. Effects of oestrous cycle control on reproductive efficiency. *Animal Reproduction Science*. 42(1-4): 307-320.

Maciel GS. 2016. Resposta superovulatória e produção in vivo de embriões em ovelhas santa inês em função da dosagem de FSH. 66f. Jaboticabal, SP. Dissertação (Mestrado em Medicina Veterinária) - Programa de Pós-graduação em Medicina Veterinária, Universidade Estadual Paulista “Júlio de Mesquita Filho”.

Matsui M, Miyamoto A. 2009. Evaluation of ovarian blood flow by colour Doppler ultrasound: practical use for reproductive management in the cow. *The Veterinary Journal*. 181(3):232–240.

Meinecke-Tillmann S. 2017. Basics of ultrasonographic examination in sheep. *Small Ruminant Research*. 152: 10-21.

Menchaca A, Rubianes E. 2004. New treatments associated with timed artificial insemination in small ruminants. *Reproduction Fertility and Development*. 16(4):403-413.

Menchaca A, Vilariño M, Crispo M. et al. 2010. New approaches to superovulation and embryo transfer in small ruminants. *Reproduction Fertility and Development*. 22(1): 113 – 118.

Menchaca A, Barrera N, Dos Santos Neto PC, et al. 2016. Advances and limitations of in vitro embryo production in sheep and goats. *Animal Reproduction*. 13(3): 273-278.

Menezes DCR, Ramos AF, Pivato I. et al. 2014. Effect of different doses of FSH on the superovulatory response in Santa Inês ewes. *Animal Reproduction*. 11(3): 348-348.

Morello HH, Chemineau P. 2008. Características Anatômicas e Funcionais do Sistema Reprodutor da Fêmea. In: Aisen EG. Reprodução ovina e caprina. 1. ed. São Paulo: MedVet. pp. 11-25.

Niswender GD, Juengel LJ, Silva PJ. et al. 2000. Mechanism controlling the function and life span of the corpus luteum. *Physiological Reviews*. 80(1): 1-29.

Noel B, Bister JL, Paquay R. 1993. Ovarian follicular dynamics in Suffolk ewes at different periods of the year. *Journal of reproduction and fertility*. 99(2):695-700.

Oliveira MEF, Cordeiro MF, Feliciano MAR. et al. 2013a. Fisiologia do Ciclo Estral. In: Oliveira MEF, Teixeira PPM, Vicente WRR. Biotécnicas Reprodutivas em Ovinos e Caprinos. 1..ed. São Paulo: MedVet. pp. 17-24.

Oliveira MEF, Feliciano MAR, D’amato CC. et al. 2014. Correlations between ovarian follicular blood flow and superovulatory responses in ewes. *Animal Reproduction Science*. 144(1-2):30-37.

Oliveira MEF, Feliciano MAR, Vicente WRR. 2013b. Ultrassonografia do Sistema Reprodutor Feminino de Pequenos Ruminantes. In: Feliciano MAR, Oliveira MEF, Vicente WRR. Ultrassonografia na Reprodução Animal. São Paulo: MedVet. pp. 141-160.

Oliveira MEF, Fonseca JF, Oliveira LG. 2013c. Transferência de Embriões. In: Oliveira MEF, Teixeira PPM, Vicente WRR. Biotécnicas Reprodutivas em ovinos e caprinos. 1. ed. São Paulo: MedVet. pp.103-120.

Pacheco A, Quirino CR. 2010. Comportamento sexual em ovinos. *Revista Brasileira de Reprodução Animal*. 34(2): 87 – 97.

Petridis IG, Barbagianni MS, Ioannidi KS, et al. 2017. Doppler ultrasonographic examination in sheep. *Small Ruminant Research*. 152: 22-32.

Pineda MH. 1989. Reproductive patterns of sheep and goat. In: McDonald LE. *Veterinary endocrinology and reproduction*. 4. ed. Philadelphia: Lea and Fabige. pp. 428-447.

Quan F, Zhang Z, An Z. et al. 2011. Multiple Factors Affecting Superovulation in Poll Dorset in China. *Reproduction in Domestic Animals*. 46(1):39-44.

Ravindra JP, Rawlings NC, Evans ACO. et al. 1994. Ultrasonographic study of ovarian follicular dynamics in ewes during the oestrous cycle. *Journal of Reproduction and Fertility*. 101(2): 501-509.

Reichenbach HD, De Oliveira MAL, Lima PF, Santos Filho AS, Andrade JCO. 2001. Transferência e criopreservação de embriões bovinos. In: Gonçalves PBD, Figueiredo JR, Freitas VJF. Biotécnicas Aplicadas à Reprodução Animal. 1. ed. São Paulo: Varela. pp. 127 – 177.

Rodriguez MGK. 2016. Dinâmica de corpos lúteos em protocolos com redução da dose de FSH na superovulação em ovelhas. 78f. Jaboticabal, SP. Dissertação (Mestrado em Medicina Veterinária) - Programa de Pós-graduação em Medicina Veterinária, Universidade Estadual Paulista “Júlio de Mesquita Filho”.

Roy F, Combes B, Vaiman D. et al. 1999. Humoral immune response to equine chorionic gonadotropin in ewes: Association with major histocompatibility complex and interference with subsequent fertility. *Biology of Reproduction*. 61(1):209-218.

Rubianes E, De Castro T, Carbajal B. 1996. Effect of high progesterone levels during the growing phase of the dominant follicle of wave 1 in ultrasonically monitored ewes. *Canadian Journal of Animal Science*. 76(3):473-475.

Rubianes E. 2000. Nociones básicas de fisiologia reproductiva em cabras y ovejas. In: Simpósio sobre controle farmacológico do ciclo estral em ruminantes. São Paulo, Brasil, p. 255-282.

Schiewe MC, Howard JG, Goodrowe KL. et al. 1990. Human menopausal gonadotropin induces ovulation in sheep, but embryo recovery after prostaglandin F2alpha synchronization is compromised by premature luteal regression. *Theriogenology*. 34(3):469–486.

Simplício AA, Freitas VJF, Fonseca JF. 2007. Biotécnicas da reprodução como técnicas de manejo reprodutivo em ovinos. *Revista Brasileira de Reprodução Animal*. 31(2):234-246.

Thompson FN. 2006. Reprodução em mamíferos do sexo feminino. In: Reece WO. Dukes - Fisiologia dos Animais Domésticos. 12. ed. Rio de Janeiro: Guanabara Koogan. pp. 644-669.

Witt MC, Bollwein H, Probst J. et al. 2012. Doppler sonography of the uterine and ovarian arteries during a superovulatory program in horses. *Theriogenology*. 77(7):1406–1414.

Wu W, Hanikezi, Yang M. et al. 2011. Effect of two follicle stimulating hormone (FSH) preparations and simplified superovulatory treatments on superovulatory response in Xinji fine-wool sheep. *African Journal of Biotechnology*. 10(70):15834-15837.

Wurst AK, Dixon AB, Inskeep EK. 2007. Lack of effect of transrectal ultrasonography with restraint on lambing rate and prolificacy in ewes. *Theriogenology*. 68(7):1012-1016.

Zaidi J, Barber J, Kyei-Mensah A. 1996. Relationship of ovarian stromal blood flow at the baseline ultrasound scan to subsequent follicular response in an in vitro fertilization program. *Obstetrics & Gynecology*. 88(5): 779–784.