



Investigação

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

**Msc Giovanna S Maciel^{1*}, Msc Mariana G K Rodriguez¹, Miss Priscila D A da Silva¹, Msc Ricardo P Nociti¹,
PhD Ricardo A R Uscategui², PhD Marcus A R Feliciano¹, PhD Wilter R R Vicente¹,
PhD Maria Emilia F Oliveira¹**

¹ Department of Animal Reproduction UNESP Univ Estadual Paulista, Jaboticabal, São Paulo, Brazil.

² Department of Surgery Veterinary, UNESP Univ Estadual Paulista, Jaboticabal, São Paulo, Brazil.

*Via de Acesso Prof. Paulo Donato Castellane s/n, Faculdade de Ciências Agrárias e Veterinárias, UNESP/Jaboticabal, CEP: 14884-900, Jaboticabal-SP. E-mail: giovanna_serpa@hotmail.com

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 PGF2α 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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