DOI: 10.21451/1809-3000.RBRA2021.029

Anais do XXIV Congresso Brasileiro de Reprodução Animal (CBRA-2021) e VIII International Symposium on Animal Biology of Reproduction – Joint Meeting, Belo Horizonte, MG, 19 a 22 de outubro de 2021.

New approaches to superovulation in goats and sheep

Novos enfoques sobre superovulação em caprinos e ovinos

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Resumo

A superovulação é a principal tecnologia reprodutiva utilizada para a produção de embriões em ovinos e caprinos, mas a imprevisibilidade dos resultados continua sendo sua maior desvantagem. Vários fatores intrínsecos e extrínsecos são responsáveis por respostas superovulatórias variáveis em pequenos ruminantes e, portanto, esta questão é o foco principal de muitos estudos que visam melhorar a produção de embriões in vivo. A raça da doadora de embriões é uma fonte importante de variação nos resultados superovulatórios, e essa variabilidade pode ser observada não apenas entre genótipos prolíficos e não prolíficos. A suplementação com progesterona usada em conjunto com protocolos superovulatórios tem efeitos benéficos no desenvolvimento folicular e na produção de embriões; no entanto, a duração ideal da exposição à progesterona também parece variar entre as raças. Alguns estudos recentes dos tratamentos superovulatórios com hormônio folículo-estimulante (FSH) abordaram o tempo para iniciar um regime, a dose total do hormônio exógeno e o número de administrações. Além disso, embora a atividade ovariana seja fortemente afetada pela estação do ano, as flutuações anuais nas respostas superovulatórias ainda são controversas e mal compreendidas. Por último, vários estudos demonstraram que a eficácia dos tratamentos superovulatórios recorrentes pode diminuir, permanecer constante ou mesmo aumentar. Esta revisão tem como objetivo discutir os principais achados em torno da superovulação em pequenos ruminantes publicados nos últimos cinco anos.

Palavras-chave: raça; tratamento com FSH; progesterona; estação; sucessivos programas de MOTE; pequenos ruminantes.

Abstract

Superovulation is the main reproductive technology used for embryo production in sheep and goats, but the unpredictability of results remains its greatest disadvantage. Several intrinsic and extrinsic factors are responsible for variable superovulatory responses in small ruminants and so this issue is the primary focus of many studies aimed to ameliorate in vivo embryo production. The breed of the embryo donor is an important source of variation in superovulatory outcomes, and such a variability can be seen not only between prolific and non-prolific genotypes. Progesterone priming used in conjunction with superovulatory protocols has beneficial effects on follicular development and embryo production; however, the optimal duration of progesterone exposure also seems to vary between breeds. Some recent studies of the superovulatory treatments using follicle-stimulating hormone (FSH) have addressed the time to start a regimen, total dose of the exogenous hormone, and the number of injections. Moreover, even though the ovarian activity is strongly affected by the season, annual fluctuations in superovulatory responses are still controversial and incompletely understood. Lastly, several studies have shown that the efficacy of recurrent superovulatory treatments can either decline, remain constant or even increase. This review aims to discuss the main findings surrounding superovulation in small ruminants and published in recent five years.

Keywords: breed, FSH treatment, progesterone, season, successive MOET programs, small ruminants.

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Recebido: 02 de setembro de 2021
Aceito: 18 de novembro de 2021

Introduction

Superovulation is a widely used technology applied worldwide to increase the individuals' offspring production, shorten inter-generation intervals, and improve reproduction rates throughout the reproductive life of ewes and does (Yao et al., 2021). The superovulatory treatment with exogenous follicle-stimulating hormone (FSH) has been used to induce growth and ovulation of multiple ovarian follicles in the same estrous cycle. This biotechnology has been employed in commercial *in vivo* embryo production as well as the establishment and expansion of germplasm banks for endangered breeds (Arrais et al., 2021), since it is the main method of viable embryo production in small ruminants (Viana, 2020).

Despite more than five decades of commercial application of multiple ovulation and embryo transfer (MOET) programs in sheep and goats, its main shortcoming is the lack of a simple, standardized, and cost-effective superovulatory protocol (Bartlewski, 2019). Superovulatory outcomes are still highly variable (Bartlewski et al., 2016; Maciel et al., 2019; Oliveira et al., 2019), making the number of viable embryos produced per donor female unpredictable. Numerous intrinsic and extrinsic factors have been implicated in variable ovarian responses and embryo production rates by different breeds of sheep and goats raised in temperate, subtropical, or tropical climates (Bartlewski et al., 2016). Even though most influencing factors or sources of variation have been minimized or eliminated, the response to superovulatory treatment remains variable, which warrants the search for strategies to optimize the existing MOET programs. This review seeks to discuss the main findings on this topic published in the last five years.

Effects of embryo donor breed

The breed of a donor is one of the main inherent factors linked to variability in superovulatory yields (Bartlewski et al., 2016). As prolificacy varies among breeds of sheep and goats, it is logical to expect that responses to an identical superovulatory treatment can also vary among different genotypes. Rebolledo et al. (2017) showed superovulatory responses were better in non-prolific Dorper ewes than in Blackbelly, Katahdin, or Pelibuey ewes (i.e., breeds with higher ovulation rates). But the differences in ovulatory responses were seen not only between prolific and non-prolific breeds sheep. Superovulated Morada Nova ewes generally had better MOET outcomes (i.e., numbers of corpora lutea (CL), recovered structures, and viable embryos) compared with Somalis Brasileira ewes (Brasil et al., 2016). Recently, our research group evaluated the superovulatory response in three naturalized Brazilian breeds of sheep (Morada Nova, Somalis Brasileira, and Santa Inês) subjected to the same superovulatory and embryo collection protocols. Superovulated Santa Inês ewes had a greater number of CL compared with Morada Nova and Somalis ewes; however, the embryo yields did not vary (Fonseca et al., unpublished data). Similar superovulatory outcomes were noted for Canindé and Moxotó goat breeds (Fonseca et al., unpublished data). Although the breed effect on superovulatory success seems to be more pronounced in sheep, the optimal superovulatory results could be obtained with the establishment of specific protocols for each breed of sheep and goats.

Progesterone/progestin-based estrus synchronization protocol

Multiple doses of exogenous FSH (superovulatory treatment) are typically applied in conjunction with an estrus synchronization protocol, which entails the pre-treatment with progesterone (P_4) or synthetic progestin for 12-14 days (long-term), 8-11 days (intermediate), or 5-7 days (short-term). Studies into P_4-based estrous synchronization protocols revealed the beneficial effects of high P_4 concentrations on follicular development and oocyte/embryonic quality, as reviewed by Bartlewski et al. (2016); however, the optimal length of the protocol seems to vary among breeds. No evident effect of P_4 priming on superovulatory outcomes was reported in Santa Inês ewes subjected to either short- (6.5 days) or long-term (14.5 days) estrous synchronization protocols followed by the 4-day or 3-day FSH treatment, respectively (Oliveira et al., 2020). Similarly, the duration of the estrous synchronization protocol (6, 9, or 12 days) had no effect on superovulatory responses and embryo production rates in non-superovulated and superovulated Morada Nova ewes, respectively (Arrais et al., 2021). In contrast, the 9-day progestagen-based estrous induction protocol was associated with higher embryo yields (i.e., numbers of total ova/embryos and of viable embryos) compared with the 6-day protocol followed by the FSH treatment in Lacaune ewes (Figueira et al., 2020a).

During the use of short-term or intermediate estrous synchronization protocols, it is necessary to include a luteolytic treatment, which can be administrated at the beginning (Fonseca et al., 2013; Camacho et al., 2019) or end of the progestogen treatment (Camacho et al., 2019; Maia et al., 2020), or at both times (Maciel et al., 2019). The administration of prostaglandin F2α at the outset of progestin treatment leads to regression of existing CL and so the ovarian status of all donor females before the superovulatory treatment is expected to be similar (Camacho et al., 2019; Oliveira et al., 2020). The effect of progesterone/progestin source in the superovulatory protocol of sheep and goats has also been studied. In Boer goats, the use of progesterone devices or fluorogestone acetate-soaked sponges for 7 days yielded the same superovulatory outcomes (Camacho et al., 2019), as previously shown in sheep receiving progesterone devices or medroxyprogesterone acetate-releasing sponges for 14-day (Bartlewski et al., 2015).

Another strategy used in association with superovulation of small ruminants is the pre-synchronization of estrus and the start of superovulatory treatment at the beginning of the synchronized estrous cycle (i.e., near the follicular wave emergence), named “Day 0 protocol”. This strategy was recently enhanced with the use of a new P4 device during the 3-day superovulatory treatment and demonstrated that high P4 concentrations during the FSH treatment led to increase in oocyte fertilization rate and embryo quality in superovulated ewes (Cuadro et al., 2018).

FSH treatment: time to start, total dose, and number of injections

The time at which the superovulatory treatment commences has been shown to be crucial for the effectiveness of MOET programs. Starting the multiple FSH injections near the time of follicular wave emergence, in the absence of a dominant follicle, leads to the best superovulatory responses. The days of follicular wave emergence during the P_4 treatment of Santa Inês ewes was influenced by the number of follicular waves and the ovarian status (i.e,. antral follicle sizes and the presence or absence of luteal structures) on day of P_4 device insertion; however, there was no seasonal effect or influence of P4 device replacement half-way through the period of treatment (Oliveira et al., 2016a). The ovulatory follicular wave in Santa Inês ewes subjected to the 8-day P_4-based synchronization protocol emerged 48 hours before the P_4 device withdrawal (Maciel et al., 2019). Using this protocol, most animals had at least one ostensibly-ovulatory sized (≥5 mm in diameter) follicle at the beginning of the superovulatory treatment. More recent studies in Morada Nova (Arrais et al., 2021) and Lacaune ewes (Figueira et al., 2020a) reported a similar ovarian follicle population at the start of FSH treatment (i.e., 60 hours before the progestin removal) in different P_4-based protocols (6, 9 or 12 days). Even though these protocols have no direct effect on the synchronization of the follicular wave, the mentioned findings indicates that the superovulatory treatment can start in a short time (60 hours) before the cessation of progesterone/progestin protocol.

In small ruminants, the strategy to begin the superovulatory treatment in the presence of only emerging follicles led to the development of the Day 0 protocol in which superovulation starts around the time of emergence of the first follicular wave of the estrous cycle (i.e., immediately post-ovulation; Cuadro et al., 2018). A single injection of estradiol during the progestin priming results in a synchronous wave emergence 4-5 days later in in anestrous ewes (Barrett et al., 2008) but not in animals during the breeding season (Bartlewski et al., 2015; Oliveira et al., 2019).

Follicle-stimulating hormone is the primary choice for superovulation and has been commonly used in dosages ranging from 150 to 300 mg per treatment cycle, either alone or combined with equine chorionic gonadotropin (eCG; Bartlewski et al., 2016). In the last decade, some studies have assessed the possibility of reducing FSH dosages in superovulatory treatments. In the Dorper ewes, Loiola-Filho et al. (2015) observed a higher fertilization rate and percentage of transferable and freezable embryos recovered from ewes treated with 128 mg of porcine FSH (pFSH) than in the ewes that received 200 mg of pFSH. In Santa Inês ewes receiving 100, 133, or 200 mg of pFSH combined with a fixed dose of 300 IU of eCG, the number of viable embryos was lower following the treatment with 133 mg compared with 100 and 200 mg of pFSH (Maciel et al., 2019). The use of 100 mg of pFSH in Santa Inês ewes was also shown to result in improved luteal development and ovarian blood perfusion as well as a lower rate of premature CL regression compared with 200 mg of pFSH (Rodriguez et al., 2019). In Lacaune ewes, the superovulatory treatment with 200 mg of pFSH was superior compared to 100 mg of pFSH (Figueira et al., 2020b). The 200-mg pFSH dose resulted in 100% of estrus responses, 92% of superovulated donors (>3 CL), and an average of seven viable embryos per donor collected (Figueira et al., 2020b). All these observations can be interpreted to suggest that the dosage of FSH selected for superovulatory treatments in sheep is also breed dependent. Choosing the most efficacious FSH dosage and applying it at the optimal time points during the progestin/progesterone-based estrous synchronization protocols appears to be a valid approach to minimize variations in superovulatory responses (Figueira et al., 2020a;b).

The duration of superovulatory treatment (that is, the number of FSH injections) can be another source of variation in the superovulatory outcomes. The 4-day ovarian superovulatory treatment regimen induced an increase in follicular blood flow, which was correlated with a negative effect on oocyte quality (number and percentage of unfertilized eggs; Oliveira et al., 2014). A direct comparison of the 3-day and 4-day superovulatory treatment regimens revealed that there was no difference in the number of embryos recovered from superovulated Santa Inês ewes; however, degenerated embryos were present only when the 4-day pFSH treatment was used (Oliveira et al., 2020).

Seasonal effects

Sheep and goats are seasonally polyestrous females with the reproductive activity induced by shortening photoperiods; however, the degree of influence of day length variations throughout the year varies between breeds and geographic location of farms (Bartlewski et al., 2011; Fatet et al., 2011). In temperate climates, there is a greater photoperiod influence on reproductive parameters than in subtropical climates, while in tropical regions the effect is generally absent.

The superovulatory outcomes in small ruminants can be strongly influenced by the seasonal shifts in photoperiod/melatonin secretion (Bartlewski et al., 2016). The growth characteristics of ovulatory follicles and estrus/ovulatory responses in estrus synchronized ewes were altered by seasonal influences under subtropical conditions (Oliveira et al., 2016b). It was suggested that even subtle differences in the stage of large follicle lifespan (regression or development) and responsiveness to gonadotropic stimuli associated with the presence or absence of luteal structures could govern the emergence of follicular waves and the ovarian status at the beginning of P_4-based protocol impinged on the days of follicular wave emergence in ewes (Oliveira et al., 2016a). Thus, season-driven variations in the follicular population at the beginning of FSH treatment could alter superovulatory responses in individual donor ewes. Differences in ovarian responses to the same superovulatory treatment can also be seen between cyclic and non-cyclic ewes treated with the combination of estradiol and progestin; such a combined treatment with steroids failed to synchronize the follicular wave emergence during the breeding season in ewes under subtropical (Oliveira et al., 2019) or temperate climate (Bartlewski et al., 2015) but it was effective in anestrous ewes under temperate climate (Barrett et al., 2008).

Comparative studies of superovulatory responses in small ruminants during different seasons are still scarce. In a recent study, the effect of seasonality (fall/winter vs. spring/summer) in ewes was apparent in the percentage of superovulated ewes, the number of CL per ewe, the recovery rate of structures, and viable embryo numbers in Santa Inês ewes in the subtropical region (Lagares et al., 2021). Conversely, the number of embryos collected and pregnancy rates after embryo transfer did not differ among the four seasons in a database study from the South region of Brazil (Bergstein-Galan et al., 2019). Boer goats raised in a temperate zone proved to be good embryo donors year-round (Camacho et al., 2019), even during seasonal anestrus.

Successive MOET programs

The use of repeated superovulatory treatments is frequently desired in sheep and goats of high genetic merit. Recent reports showed that the overall success of repeated MOET is not consistent, since the outcomes declined (Pinto et al., 2020), remained constant (Lima et al., 2015; Bergstein-Galan et al., 2019; Oliveira et al., 2020; Lagares et al., 2021) or improved over time (Figueira et al., 2020a). The main difference between the methodologies of Pinto et al. (2020) and Figueira et al. (2020a) and the other studies was the interval between successive superovulatory treatments (21 or 30 days vs. 60 days apart). Pinto et al. (2020) suggested that the shortest interval may not have guaranteed the reestablishment of luteinizing hormone (LH) reserves, which is necessary for inducing multiple ovulations; a reduction in the number of ovulations in the second and third superovulation and an increase in the rate of ovarian cysts in the third consecutive superovulation were observed. In contrast, Figueira et al. (2020a) reported greater numbers of total and vascularized CL as well as the number and rate of viable embryos in the second replicate compared with the first one, performed 30 days earlier, in Lacaune ewes. This improvement was possibly associated with the longer estrus duration in the second replicate and possibly its influence on the characteristics of the preovulatory LH peak and ovulations. Most studies mentioned above had a relatively low number of superovulatory treatments per donor (on average 2.8 collections per ewe), but in a database study, the number of recovered embryos remained similar in sheep submitted to 1, 2 to 4, or 5 to 12 MOET programs with at least one physiological estrous cycle in between (Bergstein–Galan et al., 2019).

Although the effect of repeated MOET programs may be mediated by alterations reproductive hormone secretion, an equally important consideration is related to the use of invasive surgical procedures for embryo recovery. Non-surgical embryo recovery is a safe and repeatable method in ewes resulting in a large rate of embryo recovery (Oliveira et al., 2020). The latter should therefore be a primary choice in animals undergoing multiple successive MOET procedures.

Final remarks

In superovulated sheep and goats, the breed of the embryo donor female is an important source of variation in superovulatory outcomes, and the optimal duration of the P_4- or progestin-based protocol used in combination with superovulatory treatment seems to vary between breeds. The time to start the hormonal ovarian superstimulation with FSH, the total dose of the exogenous gonadotropin used, and the number of injections are also breed-dependent and critical for maximizing the success of MOET programs, and hence require further studies. The findings on the effects of season and repeated use of superovulatory treatments in sheep and goats are still ambiguous. The applicability of the MOET programs may depend on identifying the ideal superovulatory protocols for individual breeds of sheep and goats.

Acknowledgments

The authors thank the São Paulo Research Foundation (FAPESP), the Brazilian Agricultural Research Corporation (EMBRAPA), the National Council for Scientific and Technological Development (CNPq), and the State of Minas Gerais Foundation for Research Support (FAPEMIG) by the financial support. JFF, JMGS-F, and MEFO are CNPq fellows. JFF is also a fellow of FAPEMIG and JMGS-F of FAPERJ.

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