Recent advances in superovulation in sheep

Recentes avanços na superovulação de ovelhas

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Abstract

Nearly three years ago our research team published a review of intrinsic determinants and predictors of superovulatory responses in ewes (18th International Congress on Animal Reproduction, Tours, France; 26-30 June 2016; Theriogenology 2016;86:130-143). Here's a summary of major advances in superovulatory treatments of ewes reported over the last three years and of some most anticipated future directions. The rate of genetic improvement within a synthetic population of animals is significantly enhanced by an application of superovulation and embryo transfer. However, the sheep industry has progressed rather slowly in terms of implementing these technologies in comparison to other species although sheep have been used in many studies that paved the way to the development of modern assisted reproductive technologies (ARTs). Recently, several improvements in determining ovarian responses and non-invasively collecting/transferring embryos have been reported but some most frustrating problems associated with hormonal ovarian superstimulation (e.g., unpredictable and highly variable individual responses) remain unresolved. This warrants continued studies of both the physiological basis of and novel approaches to superovulatory treatments in sheep.

Key words: sheep, superovulation, ovary, ultrasonography, transcervical embryo recovery.

Introduction

The most significant obstacles limiting widespread use of embryo transfer technology in sheep are: i. greatly limited availability of pedigree and performance data; ii. a lack of simple and cost-effective methods of superior gene dispersal in female sheep (i.e., laborious and non-standardized superovulatory protocols); and iii. the unique anatomical structure of the ewe's reproductive tract that precludes simple, transcervical passage of an insemination/embryo transfer apparatus (Candappa and Bartlewski, 2011). While technological advances greatly improved the collection and storage of animal data and breeding records, several aspects of superovulatory protocols and embryo transfer remain challenging. The application of ultrasonographic imaging in combination with hormone assays and histological studies revolutionized the study of reproductive physiology in several mammalian species including the sheep. Daily, or more frequent, transrectal ultrasonography allowed for non-invasive visualization of internal reproductive organs in conscious, non-anaesthetized animals differing in reproductive status or undergoing various hormonal treatments. This research showed that one or two large ovarian antral follicles in sheep grew in three or four groups, or follicular waves, during the interovulatory period. Several abnormalities in ovarian function, such as the failure of ovulation and luteinization of unruptured follicles, ovulations of follicles that did not result in corpus luteum (CL), CL with shortened lifespan, and ovulations of multiple follicles of varying ages and sizes were also documented (Bartlewski et al., 2011). Observations of ovarian kinetics in ewes undergoing superovulatory treatments provided an excellent opportunity to unravel the mechanisms of hormonal ovarian stimulation and can facilitate improvements to methods for superovulation (Bartlewski et al., 2008a). Recent advances in ultrasound imaging involve an application of computerized image analyses of grey-scale and color Doppler ultrasonograms (Oliveira et al., 2018a). Better understanding of endocrine mechanism surrounding cervical dilation allowed for designing more effective strategies for transcervical penetration and manipulations in ewes. In the following paragraphs, major improvements to superovulatory protocols in ewes that were achieved with the use of ovarian ultrasonography and hormone measurements have been described.

Frequency of follicle-stimulating hormone (FSH) injections

A majority of superovulatory protocols currently used in sheep utilizes exogenous FSH injections given at 12-h intervals. However, the increases in circulating concentrations of FSH after FSH doses administered twice a day may be of insufficient amplitude to consistently induce the emergence of multiple ovarian follicles. The effect of varying intervals between successive gonadotropin injections on the superovulatory outcomes in anestrous Rideau Arcott ewes were evaluated in a single study (Bartlewski et al., 2017). A superovulatory protocol wherein porcine FSH injections were given at 0800 and 1600 h was more effective in terms of inducing multiple ovulations than the protocol with 12-h intervals between consecutive pFSH doses, but it was not associated with an increased production of viable embryos by anestrous ewes.

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Advantages and disadvantages of follicle wave synchronization with estradiol/estradiol ester-progesterone/progestin priming

In anestrous ewes, a single injection of estradiol-17β (E_2-17β; 350 µg/ewe) in animals that had received medroxyprogesterone acetate (MAP)-releasing sponges induces the regression of large antral follicles and causes synchronous wave emergence ~5 days after injection. Synchronization of follicular waves with MAP and E_2-17β before superovulation reduces the variability in superovulatory responses in seasonally anovular ewes (Bartlewski et al., 2008b). However, there were no differences in superovulatory responses between Santa Inês ewes that received a single injection of 350 µg of estradiol benzoate 6 days before the first pFSH dose and their control counterparts undergoing superovulatory treatment in April (period of decreasing day lengths); all animals received 200 mg of pFSH in eight decreasing doses over four consecutive days, starting 3 days before the removal of progesterone-releasing vaginal inserts (Oliveira et al., 2018b). Moreover, variability in the proportion of degenerated embryos per donor ewe was significantly greater in EB-treated ewes. More studies are needed on the suitability of hormonal wave synchronization for superovulatory protocols in ewes at different times of the year and climatic zones.

Natural progesterone or synthetic progestin?

Cyclic Rideau Arcott x Polled Dorset ewes (November-December) received either MAP-releasing intravaginal sponges (60 mg) or controlled internal drug release (CIDR) devices (containing 300 mg of natural progesterone), with a single i.m. injection of 350 µg of E_2-17β six days after insertion of progestin/progesterone-releasing devices (Bartlewski et al., 2015). The superovulatory treatment consisted of six injections of pFSH given twice daily, followed by a bolus gonadotropin-releasing hormone (GnRH) injection (50 µg/ewe). There were no differences in ovulatory responses and embryo yields between the two groups of ewes. A decline in maximum follicle size after an E_2-17β injection was more abrupt in CIDR®- compared with MAP-treated animals, and the ewes receiving CIDR® had significantly more small antral follicles (3-mm in diameter) at the start of the superovulatory treatment. In both subsets of animals, the next follicular wave emerged ~2.5 days after E_2-17β injection. Serum concentrations of exogenous E_2-17β were lower in MAP-treated ewes. Despite these differences in antral follicle numbers and estrogen concentrations between MAP- and CIDR®-treated cyclic ewes, there were no differences in superovulatory responses and embryo yields.

Long vs. short superovulatory protocols

Most of the superovulatory protocols currently used are lengthy and cumbersome but there is a paucity of studies directly comparing the outcomes of long- and short-term superovulatory treatments in ewes. In a recently conducted experiment, Oliveira et al. (unpublished) compared the superovulatory results between the ewes subjected to a long-term progesterone combined with a 4-day multiple-dose pFSH regimen (14.5-day protocol) and animals receiving pFSH injections over 3 days following a short-term P_4 pre-treatment (6.5-day protocol); a total dose of 200 mg of pFSH was used in both groups of animals. Embryos were recovered 6 to 7 days after the onset of estrus by the transcervical uterine flushing. There were no differences between the two groups of ewes in the duration of or degree of difficulty associated with uterine flushing; the average duration of cervical penetration preceding embryo flushing was 5.6 ± 0.8 min (range: 1 to 17 min) and the average duration of the uterine flushing was 28.5±1.0 min (range: 21 to 40 min) with the mean fluid recovery of 96.3±0.8% (range: 78.5 to 100%). Superovulatory yields did not vary between the two protocols except for the mean number of degenerated embryos that was greater for the 6.5-day treatment group compared with the 14.5-day protocol.

What is the best time to start superovulatory treatments?

Superovulatory protocols (e.g., exogenous FSH injections) initiated at or around the time of follicle wave emergence yield better results that superovulatory treatments started at later stages of follicle wave development. Oliveria et al. (2016) reported that the long-term (14 days) estrus synchronization treatment with CIDR is associated with a distinctive pattern of follicular wave dynamics in ewes kept in subtropical climate. Follicular wave emergence throughout the period of CIDR application was affected mainly by the number of emerging follicular waves/season and ovarian status at CIDR insertion. For example, the presence of medium-sized antral follicles (4.0 to 5.75 mm in diameter) in the absence of corpora lutea (CL) at the time of CIDR insertion advanced follicular wave emergence. Therefore, superovulatory treatments should take into consideration the ovarian status of ewes at the time of CIDR insertion (i.e., size of antral follicles and presence of luteal structures) and they should commence on Days 5 or 9 of CIDR treatment in ewes without CL and ovaries bearing medium-sized follicles; these days correspond to emergence of the 2nd and 3rd consecutive wave of follicle growth in that group of acyclic Santa Inês ewes as opposed to Days 6 and 10 in remaining animals. Another aspect that can impinge on the ovarian response to superovulatory treatments is the number of small follicles, especially that of FSH-responsive follicles, present at the time of exogenous FSH injections. The influence of exogenous progesterone/progestin on the populations of non-atretic small antral follicles in ewes at different times of the year and maintained under different climatic conditions remains to be elucidated.

Predicting superovulatory results with B-mode and color Doppler ultrasonography

At present, the collection of embryos from superovulated donor ewes is typically accomplished with laparotomy and although this technique offers the highest embryo recovery rates it remains an invasive and traumatic procedure. Therefore, it should only be performed in animals that responded well to hormonal ovarian stimulation. The objective of the recent study by Oliveira et al. (2018a) was to assess the usefulness of two B-mode and color Doppler sonography, and serum progesterone (P_4) measurements for determining the ovarian response in superovulated ewes. Six days after natural mating the total number of ultrasonographically detectable CL and luteinized unovulated follicles were strongly and positively correlated with those detected with videolaparoscopy and circulating P_4 concentrations were related directly to the number of healthy CL; the use of color Doppler appeared to increase the accuracy of CL detection and enumeration. More research is needed to identify prematurely regressing CL with the ultrasonographic technology as they could not be distinguished from healthy CL using visual assessment of either B-mode or color Doppler ultrasonograms.

Transcervical embryo recovery and transfer

Minimally invasive embryo flushing and transfer via transcervical route is arguably one of the most significant advances in popularizing commercial multiple ovulation and embryo transfer (MOET) programs in ewes. There is increasing evidence that non-surgical embryo recovery is a valid alternative to the surgical methods in small ruminants. Several drugs have successfully been applied to dilate the uterine cervix for transcervical embryo flushing and deposition, with a combined treatment with estradiol benzoate, d-cloprostenol (both administered 16 h before embryo flushing) and oxytocin (20 min prior to cervical penetration) providing the most optimal results in cycling and superovulated ewes (Fonseca et al., 2019). More studies are needed to determine the effects of sheep genotype, age and reproductive season as well as an application of anti-inflammatory drugs post-treatment on the efficacy of the cervical penetration procedures in sheep.

Concluding remarks

Inclusion of assisted reproductive technologies (ARTs) in animal husbandry is the most effective approach to increasing livestock genetics and productivity traits. By comparison to large ruminants, swine and poultry operations, the sheep industry has progressed rather slowly in terms of application of ARTs but recent developments in application of superovulatory protocols and low-invasions transcervical manipulations promise to results in popularization of these techniques in commercial settings and biotechnology research.

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