Small Ruminant Research 136 (2016) 54–58

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Superovulatory and embryo yielding in sheep using increased exposure time to progesterone associated with a GnRH agonist

O.O. Brasil[a,b], N.H. Moreira[a,b], G. Santos Júnior[a,b], B.D.M. Silva[b], A.S. Mariante[b], A.F. Ramos[b,*]

[a] Graduate Course on Animal Sciences—College of Agriculture and Veterinary, University of Brasilia, Brasilia, Brazil
[b] Embrapa Genetic Resources and Biotechnology, Brasília, Brazil

ARTICLE INFO

Article history:
Received 1 August 2015
Accepted 6 January 2016
Available online 11 January 2016

Keywords:
Biotechnology
Conservation
Reproduction
Superstimulation
Ewe

ABSTRACT

Response to superovulatory, embryo yields and quality, were evaluated after increasing exposure time to exogenous progesterone during superstimulation, with or without the addition of a GnRH agonist. Thirty-four ewes from the Santa Inês breed were synchronized with an intravaginal progesterone device (CIDR). The animals were randomly divided into three groups: exposure to progesterone for 14 days (Control, $n = 12$); 12 h extension of progesterone exposure (12hP4 group, $n = 11$); and 12 h extension, associated with the administration of 25 μg of gonadorelin acetate (12hP4GnRH group, $n = 11$). From the 12th to the 15th day of protocol, 133 mg of pFSH were administered through eight decreasing doses. Artificial inseminations were performed 36 and 48 h after device withdrawal, using frozen/thawed semen. The number of ewes on estrus and the time to onset of estrus after withdrawing device were similar among between all groups ($P > 0.05$). All groups presented high superstimulatory response (averages ranging from 14.33 to 16.18 follicles ≥4 mm) ($P > 0.05$). All groups also showed a large quantity of CL (averages ranging from 11 to 12) ($P > 0.05$). The longer exposure to progesterone no reduced degenerated embryo proportion (Control = 30%; 12hP4 = 7% and 12hP4GnRH = 10%; $P > 0.05$). The fertilization rate was significantly higher in 12hP4GnRH when compared to Control and to 12hP4 (77%, 34% and 41% respectively). These results suggest the association of longer progesterone exposure plus GnRH administration is an alternative to increase oocyte fertilization rates when fixed time insemination is used.

1. Introduction

Multiple ovulation and embryo transfer (MOET) technologies have substantial contributions to genetic improvement of sheep in several countries around the world. During the last three decades there has been considerable progress in the use of female germplasm through MOET. However, this technology applied to sheep has been of slow acceptance, mainly due to the oscillation of superovulatory (SOV) response (Cognie, 1999; Cognie and Baril, 2002). The main factors which influence variation in results are type of superstimulatory treatment, season, follicular condition, genetics and nutritional status of animals (Ammoun et al., 2006; Gonzalez-Bulnes et al., 2003). These factors can be directly or indirectly involved, thus influencing the oocyte/embryo quality and/or synchronization of multiple ovulations.

By the end of the superestimulatory treatment, oocytes at different stages of development are present in large follicles. Therefore, an attempt to permit such oocytes to acquire equal competence is necessary in order to achieve in higher production of good quality embryos. This could be achieved by a prolonged exposure to progesterone, with the aim to delay the LH peak and cause synchronized ovulation. Moreover, the use of the gonadotropin releasing hormone (GnRH) or its agonists, after FSH stimulation, provides better synchronization of ovulation, increasing embryo yields (Menchaca et al., 2009; Walker et al., 1986), in spite of controversial reports (Baril et al., 1996; Jabbour et al., 1996). We tested the hypotheses that prolonged exposure to progesterone (1) increase the synchronize of the ovulations, or (2) when used longer progesterone exposure plus GnRH further improves the ovulation synchronize, increasing the fertilization rate of the ewes inseminated with frozen/thawed semen in MOET programs.

Therefore, a longer progesterone exposure associated or not to ovulation induction, seems to be a viable alternative to increase

* Correspondence to: Parque Estação Biológica, Av. W5 Norte Final, Asa Norte, 70770–917 Brasília, Brazil. Fax: +55 61 33403669.
E-mail addresses: alexandre.floriani@embrapa.br, aleframos@yahoo.com.br (A.F. Ramos).

http://dx.doi.org/10.1016/j.smallrumres.2016.01.005
0921-4488/

oocyte viability and fertilization rate, especially when fixed time insemination with frozen/thawed semen is used. The aim of this study was to evaluate response to superovulatory, embryo yields and quality, using frozen/thawed semen, after an increased exposure time to exogenous progesterone during superovulation, with or without the addition of a GnRH agonist.

2. Materials and methods

This experiment was approved by the Animal Ethics Committee (CEUA) of the Institute of Biological Sciences at the University of Brasilia.

2.1. Experimental station and animals

This study was developed during the period of May to July, 2012 in Brasilia, located on the Central-West region of Brazil (latitude 15°52′S, longitude 48°00′W), with an altitude ranging from 1050 to 1250 m above sea level. This region presents a tropical rainy climate, with marked dry winters and rainy summers. Thirty-four ewes from the Santa Inês breed, with a body condition score of 2.9 ± 0.3 (range 1–5; Russel et al., 1969) and body weight of 47.4 ± 6.6 kg were used as embryo donors. Santa Inês breed has cyclical behavior during the year at this location. The animals were kept in a pasture of *Panicum maximum*, and had free access to water and mineral salt.

2.2. Treatments

All animals were synchronized with an insertion (Day 0) of a progesterone controlled-internal-drug-release device (Eazi-Breed CIDR—Controlled Internal Drug Release, Pfizer, New Zealand) for 14 days, with a new controlled-internal-drug-release introduced at Day 7. During the exchange of the CIDR, on Day 7, a luteolytic dosis of PGF2α (37.5 mg d-cloprostenol; Prolise, Tecnopec, ARSA SRL, Argentina; im) was administered. On Day 12, the superstimulation was induced using 133 mg of pFSH (Folltropin, Tecnopec, AHC Inc., Bioniche, Canada; im) that was administered through eight decreasing doses, twice a day, starting in the morning of Day 12 and finalizing in the afternoon of Day 15. At the time of CIDR removal, the ewes were randomly divided into three experimental groups: 1. Control ($n = 12$): progesterone device withdrawn at Day 14; 2. 12hP4 group ($n = 11$): progesterone device maintained for 12 more hours, until Day 14½; and 3. 12hP4GnRH group ($n = 11$) progesterone maintained for 12 more hours, until Day 14½ plus GnRH agonist (25 μg of Gonadorelin acetate; Gestran Plus, Tecnopec, ARSA SRL, Argentina; im) administered simultaneously with the last pFSH injection (Fig. 1).

2.3. Evaluation of estrus and artificial insemination

Estrus was observed at intervals of four hours counting from moment of CIDR withdrawal, until the moment of first insemination, with the aid of a teaser ram that had his pectoral region painted with a mixture of pigment powder and soybean oil. The ewes that were considered in estrus were those that had paint on their back and accepted mounting. All females were artificially inseminated with frozen/thawed semen via the laparoscopic method delineated by Maxwell and Butler (1984). The AI was preformed 36 and 48 h after CIDR withdrawal, using straws of 0.25 mL containing an insemination dosis of 100×10^6 spermatozoa, with parameters established by the Colégio Brasileiro de Reprodução Animal (1998). Half of each semen dose was introduced into each uterine horn by the laparoscopic method.

2.4. Superstimulatory response

Two ultrasound evaluations of ovarian activity were performed on all females, using the B-mode real-time scanner (Aloka Echo Camera SSD 500, Overseas Monitor Corp., Ltd., Richmond, BC, Canada) and an adapted 7.5 MHz transducer for transrectal exams. The first evaluation was preformed after the last FSH injection, to assess the superstimulatory response at the end of the gonadotropin treatment. The second evaluation was performed 12 h after the last AI, to assess the amount of large follicles that had not ovulated yet. All follicles larger than 4 mm were counted and drawn on maps.

2.5. Ovulatory response and embryo yield

On Day 7 after pessary removal, the number of ovulations was recorded by laparoscopic procedure and, immediately afterward, embryos were surgically recovered under general anesthesia. Ewes, deprived of food and water for 24 h, were im administered with xylazine (1.1 mg/10 kg; Rompun, Bayer, Brazil) and ketamine hydrochloride (20 mg/10 kg Ketamina, Agener, Brazil). Furthermore, local anesthesia was administered in the surgical field (0.2 mg of lidocaine; Lidovet, Bravet, Brazil). Ova/embryos were collected surgically after ventral laparotomy, using a paramedian incision (6 cm long) cranial to the udder to access the reproductive tract. Each uterine horn was flushed with 60 mL flushing media (DPBS, Cultilab, Brazil) which was collected via a 9 FG Foley catheter, inserted at the external bifurcation of the uterine horns. Embryos were recovered in a Petri dish, maintained in holding media (Holding plus, 0.4% BSA, Embriocare, Cultilab, Brazil), and morphologically evaluated under a stereomicroscope (×40 magnification), following the International Embryo Transfer Society (IETS) recommendations (Robertson and Nelson, 1999). The quality score of the embryos were: Grade 1 (excellent or good), Grade 2 (fair), Grade 3 (poor), and Grade 4 (dead or degenerated). Embryos graded as 1, 2 and 3 were considered viable and those were graded 1 and 2 were considered freezable embryos.

2.6. Indicators of superovulatory response

The following data were recorded for each ewe: number of corpora lutea (CL), total recovered oocytes/embryos (TR), viable embryos (VE), freezable embryos (FE), degenerated embryos (DGE), and total embryos (TE). The fertilization rate (FR) was obtained by dividing TE by TR; the freezable embryo rate (FER) was obtained by dividing FE by VE; while the degenerated rate (DGER) was obtained by dividing DGE by TE.

2.7. Statistical analysis

The data were analyzed using the SAS software (V9, SAS Institute Inc, Cary, NC, 2003). The parameters related to the amount of follicles during the last FSH injection and 12 h after AI, quantity of CL and total recovered structures were subjected to an analysis of variance (ANOVA) and the Duncan's test, to compare their means. Variables that did not a normal distribution, such as the number of viable and freezable embryos, have been evaluated using the Kruskal–Wallis test. Percentage of ewes exhibited estrus and embryo degeneration rate were analyzed by the Fisher exact test, while fertilization and freezable embryos rate were analyzed by the Chi-square test. Results are shown as Mean ± Standard Deviation or in percentage, and the differences were considered significant when $P < 0.05$.