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Conceptus loss in Santa Inês ewes carrying twin pregnancies by natural mating or embryo transfer

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ABSTRACT

Commercial application of reproductive biotechnologies such as multiple ovulation and embryo transfer depends on its overall efficiency. Sheep embryo transfer is gradually gaining wider adoption, but pregnancy rates after embryo transfer remain lower than those derived from natural mating for most breeds. The work was aimed to evaluate embryonic and fetal losses in Santa Inês ewes carrying twin pregnancies by natural mating or embryo transfer. Ewes were subjected to synchronized natural mating by ram effect or used as recipients for embryo transfer. Ewes diagnosed as carrying twin pregnancies at day 25 were used in the experiment (n = 42). Conceptus viability was monitored by ultrasonography on days 30, 35, 40, 45, 50, and 55 after conception. Conceptus loss was similar (P > 0.05) within natural mating 11/42 (26.19%) and embryo transfer 14/42 (33.34%). However, overall embryonic loss (80.0%) was greater (P < 0.05) than fetal loss (20.0%), with no difference within groups The results allow the conclusion that conceptus loss after embryo transfer is similar to natural mating and occurs predominantly during the embryonic stages.

1. Introduction

The adoption of adequate reproductive biotechnologies can contribute to increase herd reproductive performance of valuable animals, suggesting that sheep reproductive potential remains not fully explored [1–3]. However, among many factors that may negatively influence reproductive efficiency, conceptus loss during both embryonic and fetal development is the major cause of reproductive losses in farm animals [4–6]. These losses interfere in the reproductive performance by prolonging lambing intervals and diminishing prolificacy [7].

Early reports showed that ewes with two ovulations might retain two embryos, one embryo or none [8]. Thus, the death of a single embryo in twin or triplet pregnancies may not lead to total pregnancy loss [9,10]. Sheep multiple pregnancies are more resistant to such losses than cattle, due to embryo migration during early development, ensuring adequate uterine occupancy by conceptuses [11]. Most reports on embryonic loss described it until day 30 of pregnancy [9,10,12–14], while losses after this period were somewhat limited [9,10,14].

Since most pregnancy losses occur during its establishment [10,14–16], early diagnosis is of fundamental importance to minimize reproductive losses by rapid identification of non-pregnant ewes [5]. Embryonic loss is correlated with conceptus live weight during early pregnancy [17]. These losses may be caused by environmental factors [17–23], genetic or embryonic factors [24–26], management practices [24], inadequate usage of reproductive biotechnologies [19], among others [27,28]. Despite these factors that may contribute to embryonic loss, incomplete conceptus to maternal signaling crosstalk is suggested as the major factor for pregnancy loss [27,29,30].

There is evidence that ewes with multiple embryos/fetuses display greater a propensity for embryonic loss, possibly due to both restricted uterine space and nutrient supply [6,10,31]. Despite earlier reports on conceptus loss in ewes [5,30], to our knowledge,

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there are no reports focusing on embryonic and fetal loss from twin pregnancies established by natural mating and embryo transfer. Under this context, the investigation of twin pregnancies alone could better define the effect of embryo source on conceptus viability. Thus, the work was aimed to evaluate embryonic and fetal losses in Santa Inês ewes carrying twin pregnancies by natural mating or embryo transfer.

2. Material and methods

2.1. Animal management

Experiments were conducted in Escada, Pernambuco state, Brazil. Geographic coordinates are latitude 08° 21′ 33″ S, longitude 35° 13′ 25″ O, an altitude of 109 m, with a mean annual temperature of 24.4 °C and mean annual rainfall of 1,763 mm[3].

Animals were raised under pasture conditions during the day and kept in pens during the night. Pastures were composed of cultivated species (*Brachiaria humidicola and Brachiaria tunnergrass*) and native species (*Paspalum maritimum, Chloris orthonton, Cynodon dactylon*). Animals were also fed with *Pennisetum purpureum* when kept in pens. Mineral salt and water were offered *ad libitum*.

Multiparous Santa Inês ewes (n = 133) of 2–4 years of age and body condition score from 2.5 to 3.5 (on a 1 to 5 scale) of proven fertility (without previous reproductive problems) were used in the natural mating or embryo transfer groups.

Santa Inês rams of 3–5 years of age (n = 8), body condition score from 3.0 to 3.5 (on a 1 to 5 scale) and proven fertility were used. One week before the onset of the experiment, rams were subjected to andrological evaluation. These animals were selected based on their general clinical evaluation, normal genital system, and a spermogram above minimum limits stipulated by the Brazilian College of Animal Reproduction: progressive sperm motility (minimum of 80%), sperm vigor (3 or greater), and total defective cells (<20%).

2.2. Natural mating

The ewes (n = 81) were subject to the ram effect, in order to induce and synchronize estrus [1]. They were initially isolated from males for 35 days for ram effect preconditioning. After this period, vasectomized rams (n = 9) using chin ball marker were introduced into ewe flocks. The estrus observation was performed twice a day by identification of ewes marked with ink (6:00 to 7:00 and 17:00 to 18:00 h). Ewes scored in estrus were taken to the fertile rams (n = 6) for mating.

2.3. Embryo transfer

Embryo donors (n = 11) had their estrous cycles synchronized by insertion of vaginal devices impregnated with 0.33 g of natural progesterone, controlled internal drug release (CIDR, Pfizer, Auckland, New Zealand), and considered it day 0 on protocol (D0). On day 9, all CIDR were replaced by new devices and were used until day 13. The FSH-treatment was initiated on day 11 until day 15, using 252 mg of follicle stimulating hormone - pFSH (Folltropin-V, Bioniche, Ontario, Canada) with intramuscular (IM) shots, divided in eight decreasing doses (46 mg, 36 mg, 24 mg, and 20 mg twice a day), administered in 12-h intervals. Concomitant with the removal of vaginal dispositive on day 13, 200 IU de equine chorionic gonadotropin (eCG) was administered (Folligon, Intervet, Boxmeer, Holland). Controlled natural mating was performed on day 14 with rams of proven fertility.

Expanded blastocysts (n = 57) were collected on day 6.0 after the onset of estrus. Females were not fed 24 h before collection and were anesthetized with 0.2 mg kg^{-1} xylazine chloride (Rompun, Bayer, São Paulo, Brazil) and 7.5 mg kg^{-1} ketamine chloride (Ketalar, Parke-Davis, Buenos Aires, Argentina) by IM shots. Embryo collections were performed by laparotomy, and both uterine horns were flushed with embryo collection medium Dulbecco's modified phosphate buffered saline (DPBS, Embrio care, Cultilab, Campinas, Brazil), supplemented with 1% fetal bovine serum (FBS) at 37 °C. Embryos were immediately identified and placed in holding medium (TQC Holding Plus, Nutricell, Bioniche, Athens, USA). Embryos were classified according to the stage of development and quality as proposed by the IETS manual [32], and only embryos scored as grade I were selected for transfer.

Santa Inês recipient ewes (n = 28) were synchronized using a vaginal device with 60 mg of medroxyprogesterone acetate (Progespon, Syntex, Buenos Aires, Argentina) for 14 days followed by 400 IU eCG concomitant with device removal (protocol was initiated on Day −1 of the donor ewe protocol). Twenty-four hours after device removal, vasectomized males were used for estrus detection. Embryo transfer was performed by laparoscopy on day 7 after estrous detection. Recipient ewes were anesthetized with 0.05 mg kg^{-1} xylazine chloride (Rompun, Bayer, São Paulo, Brazil). The uterine horn ipsilateral to the corpus luteum was exposed, and two blastocysts were transferred with a tomcat catheter (Nutricell, Bioniche, Athens, USA).

2.4. Pregnancy diagnosis and conceptus monitoring

Pregnancy diagnosis was carried out by transrectal ultrasonography (Áquila-Pró, Esaote Pie Medical®-Maastricht/Holland) using a linear transducer (6.0 and 8.0 MHz) adapted on a PVC support to facilitate manipulation in the rectum of the animal [4]. Ewes subject to natural mating were pregnancy diagnosed on the 25th day after the mating. Recipient ewes were diagnosed on the 19th day after embryo transfer (25th day of pregnancy). Pregnancies were determined by embryo visualization. Only ewes diagnosed as carrying twin pregnancies were used in the experiment.

2.5. Conceptus loss evaluation

The embryonic and fetal loss evaluation, carried out only on females diagnosed with twin pregnancy (natural mating - n = 21; recipient ewes - n = 21), was monitored in five-day intervals (30, 35, 40, 45, 50, and 55) after pregnancy diagnosis. All ultrasound examinations were performed by the same technician, and physical ultrasound images were generated on a Sony printer (Seikosha VP/1200 - Tokyo/Japan) to keep a record of all pregnancies. Conceptus loss was determined by the absence of heartbeat, presence of fetal malformations, reduction in amniotic fluid content or lack of fetal movement, echogenicity [4]. The embryonic loss was described as those that occurred up to day 34 (ultrasonography examinations at days 30 and 35), while the fetal loss was those from day 35 onwards [33].

2.6. Statistical analysis

The conceptus loss was analyzed by the chi-square or Fisher's exact test, and prolificacy was determined by unpaired T-test, with a significance level of 5%.

3. Results

A total of 21 out of 28 recipents were confirmed for twin pregnancies after embryo transfer (75.0%). From the 81 ewes subjected to natural mating, 72 ewes became pregnant (88.8%). From those