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Early embryo loss, morphology, and effect of previous immunization against androstenedione in the ewe

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ABSTRACT

In a naturally mated cycle, ova and viable embryo number as well as embryo size were assessed on Day 4, 10, 14, 18, and 30 of gestation in Romney ewes (n = 38–44 per gestational group). For Days 4–18 of gestation, embryos were recovered by flushing the reproductive tract after slaughtering of the ewe. Ovulation rate was determined by counting the number of corpora lutea present on both ovaries. For the Day 30 group, number of ovulations was measured by laparoscopic examination of the ovaries at Day 9–12 of the cycle, and number of embryos present was determined by ultrasound examination at approximately Day 30 of pregnancy. Most of embryo loss occurred before Day 14 of gestation with 6% loss before Day 4, and 12% loss between Day 4 and 14 of gestation. A similar proportion of viable embryos per number of ova ovulated were recovered on Day 14 and 18 (82%) and Day 30 (81%) of gestation. Fertilization failure was estimated at 1%. Conceptus and embryo size was most variable on Day 14, representing a period of rapid growth (conceptus length ± standard deviation); Day 4 (169 ± 8 μm), Day 10 (379 ± 93 μm), Day 14 (23 ± 32 mm), Day 18 (embryo length ± standard deviation; 5.0 ± 0.7 mm). Vaccination with commercially available fertility vaccines targeting androstenedione (Androvax and Ovastim) in previous seasons resulted in reduced conceptus size compared with controls. However, no difference in the proportion of viable embryos was observed between treatments, signifying maternal tolerance for considerable variation at this stage of development. Furthermore, the finding that most of loss occurs within the first 14 days of gestation highlights the importance of both oocyte quality and the uterine environment for the embryo to successfully overcome the challenges leading up to and including pregnancy recognition in the ewe.

1. Introduction

Reproductive rate is a major limitation of livestock production efficiency. Considerable improvements in the reproductive rate of ewes have been made in recent decades, primarily through increased ovulation rate by introgression of Inverdale and Booroola genetics [1], genetic selection for number of lambs born [2], vaccination against androstenedione (Androvax, Ovastim [3]), or improved management of nutrition for reproductive success [4]. However, in ewes more than 30% of ova are lost by Day 30 of gestation [5–7], representing a substantial opportunity cost to sheep farming. As a first step to providing solutions for overcoming this loss, more detail is needed about the challenges the developing embryo faces in the modern farm environment.

In sheep, onset of estrus occurs 7 to 9 hours before the LH surge which is then followed by ovulation 20 to 28 hours later [8,9]. Estrus behavior persists for approximately 24 to

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36 hours. Timing of these events can be influenced by synchronization methods such as prostaglandin treatment and progesterone pessaries [8]. Semen deposited by the ram at the neck of the cervix reaches the caudal isthmus of the oviduct within hours of mating [10]. Viable sperm adhere to the wall of the caudal isthmus, whereas nonviable sperm are voided through the oviduct and into the peritoneal cavity ahead of ovulation [10]. A local and temporal surge of progesterone at ovulation stimulates release of the hyperactivated sperm from the caudal isthmus into the oviductal lumen where fertilization takes place [5]. Cleavage occurs within 18 to 36 hours of fertilization and, at least *in vitro*, embryos undergoing cleavage earlier have increased viability compared with those undergoing cleavage later [11]. The embryo undergoes three more cell divisions of approximately 22.5 hours/division [12] which increase cell number, but not total cytoplasm volume, to form a 4-cell blastomere, an 8-cell blastomere, and then a 16-cell morula contained within the original oocyte zona pellucida and located in the oviduct. Embryonic genome activation occurs at the 8-cell stage in sheep [13].

After compaction of the morula, the next step is the formation of the blastocyst involving the formation of a fluid cavity, termed the blastocoel, and differentiation of the epiblast (which will develop into the embryo) from the trophoblast (which will develop into the placenta). This stage also coincides with the passage of the embryo into the uterus (Day 4–5 of gestation) through the utero-tubal-junction. In the uterus, the ovine blastocyst hatches from the zona pellucida (Day 8) and expands. The trophoblast becomes ovoid, then tubular (Day 11), then lengthens (up to 10 cm by Day 14), and becomes filamentous in shape [14]. At this stage, the embryonic trophoblast must produce and secrete sufficient interferon-tau into the histotroph before Day 13 of gestation to ensure maternal recognition of pregnancy [15]. The consensus of research activity in previous decades concluded that early embryo mortality was substantial ($\geq$30% mortality in first 30 days), and occurred primarily before implantation (Day 18 in sheep) [7], whereas late fetal loss (Day 60 to term) was low ($<$5%) in the absence of disease or stress [16,17]. Fertilization failure is low in sheep (1%–2%), and embryo loss due to chromosomal abnormalities is also relatively low (6%) [18]. The aims of the present study were to describe the natural variety of embryo morphology and further define the critical period(s) in which most of embryo loss is observed in the modern productive ewe, including the effect of previous immunization against androstenedione.

2. Materials and methods

In accordance with the Animal Welfare Act Regulations of New Zealand, prior approval for the experiment was given by the Animal Ethics Committee of Invermay Agricultural Centre, where the study was conducted (45°51 S, 170°23 E) commencing in autumn.

2.1. Animals

Parous (4.5 years old) Romney ewes with a mean (±standard error of the mean) live weight of 66.8 ± 0.5 kg were kept on ryegrass/white clover pasture. The ewes were randomly allocated into five groups, balanced for live weight, and previous treatment with commercially available fertility vaccines (Ovastim, Androvax, and Control). The treatment with commercially available fertility vaccines had occurred over the previous three breeding seasons, with the last booster injection given approximately 12 months before this study. The vaccines were given following manufacturers' instructions as described [3]. These groups were assessed at key gestational milestones (n = 38–44 per time point): morula formation (Day 4), ovoid/expanded blastocyst development (Day 10), trophoblast elongation (Day 14), implantation (Day 18), and embryo establishment (Day 30) after which losses are expected to be low [7].

2.2. Estrus synchronization and mating

To mitigate potential confounding effects of hormone treatment on embryo development, ewes were mated by fertile rams on the second cycle after estrus synchronization induced using intravaginal progesterone releasing devices (0.3 g progesterone; CIDR-G, Pfizer, Auckland, New Zealand) inserted for 12 days. After CIDR removal, estrous activity was detected using harnessed vasectomized rams (two rams per approximately 45 ewes) with ewes checked daily (am) for mating marks over 4 days. From 15 days after CIDR removal, the ewes were divided into three mobs according to the day of pregnancy that they were to be assessed at: Day 10 and Day 30 (mob1), Day 14 and Day 18 (mob two), and Day 4 (mob three) of gestation. Mobs were exposed to harnessed Romney rams of proven fertility (3 rams per approximately 90 ewes [mob 1 and 2], two rams per approximately 45 ewes [mob 3]). Mating marks were recorded daily (am) for up to 8 days with the first day that a fertile ram mating mark was observed recorded as Day 1 as mating will have occurred in the previous 24 hours. Ewes were mated by fertile rams between 16 and 23 days after CIDR removal (average cycle length 18.7 ± 0.1 days).

2.3. Collection of embryos and measurement of ovulation rate on Day 4–18

For Day 4–Day 18 time points, ewes were slaughtered by captive bolt and exsanguination, reproductive tracts recovered and flushed with sterile Dulbeccos PBS with 0.1% polyvinyl alcohol (both Sigma–Aldrich Ltd., Auckland, New Zealand) to obtain embryos. Ovulation rate of ewes (determined from the number of CL on the ovaries), as well as number, size and quality measurements of embryos recovered, were recorded postmortem.

2.4. Embryo assessment on Day 4–18

Recovered embryos were counted and assessed for fertilization, quality and stage of development, and photographed using either a Leitz labolux K compound microscope (Leica Microsystems, Vienna, Austria) or an Olympus SMZ1000 stereo microscope (Coherent Scientific, Auckland, NZ). Developmental stage (Table 1) and quality of