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An earlier rise in systemic progesterone and increased progesterone in the uterine vein during early pregnancy are associated with enhanced embryonic survival in the ewe

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ABSTRACT

Improved livestock production efficiency through greater embryonic survival (ES) is of economic and animal welfare benefit. Physiological characterization of animals that are extreme outliers for ES provides a valuable opportunity to identify a naturally occurring mechanism by which this trait may be enhanced. The objective was to determine the likely cause for the lifetime history of enhanced or reduced ES in a line of ewes selected for high fecundity. To address this question, progesterone concentrations in peripheral plasma as well as ovarian and uterine venous plasma samples were compared between groups of ewes with a lifetime history of either enhanced or reduced ES. The ability of the uterus to synthesize progesterone *de novo* at Day 5 of gestation was also tested. Ewes with enhanced ES had an earlier rise in progesterone concentration after estrus, irrespective of pregnancy status. In addition, there were increased concentrations of progesterone in the uterine vein in enhanced ES compared with reduced ES ewes on Day 5 of gestation (8.3 ± 0.8 ng/mL and 3.9 ± 1.4 ng/mL, respectively, $P < 0.05$). However, there were no differences in ovarian venous plasma (enhanced ES, 1725 ± 166 ng/mL; reduced ES, 1665 ± 268 ng/mL) at Day 5 of gestation. Although the endometrial tissue of some ewes (3/8) at Day 5 of gestation expressed three of the key genes necessary for regulation of *de novo* synthesis of progesterone, expression was not present exclusively in either of the two ES groups and therefore was unlikely to explain differences in the uterine vein progesterone concentrations between the enhanced and reduced ES groups. Collectively, the earlier rise in progesterone concentrations in peripheral plasma during the first week of gestation in the enhanced ES animals was independent of the presence of an embryo. Moreover, increased progesterone concentrations were also observed in the uterine vein at Day 5 of gestation of the enhanced ES ewes. It is proposed that the difference in uterine vein progesterone concentration was likely due to the differences in ovarian venous blood supply rather than *de novo* synthesis by the uterus.

1. Introduction

In many species, including sheep, >30% of ovulated ova fail to develop to term [1–3]. In the livestock industries, an improvement in this aspect of reproductive performance would represent a profound increase in production

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efficiency. In naturally mated New Zealand Romney sheep, estimates for fertilization failure (8.7% of eggs ovulated), early embryo failure (30% of potential embryos up to Day 30 of gestation), and mid-pregnancy failure (<5% of potential lambs between Days 30 and 140 gestation) were reported by Quinlivan et al. [4]. Although greater embryo loss in other breeds of sheep from synchronized estrous cycles were reported by Dixon et al. [5], we propose that most reproductive loss occurred between fertilization and Day 30 of gestation in natural breeding situations.

The contributions of nutrient supply [2,6], hormonal milieu including progesterone [7,8], disease and physical accommodation of the growing conceptus, to pregnancy have been investigated extensively. However, the maternal influence on survival of the individual embryo, especially in ewes that consistently have multiple versus single births after multiple ovulations, is not well understood. As a trait, embryonic survival (ES) has previously been reported to have low heritability in sheep [9]. Hanrahan et al. [9] developed an equation for predicting average litter size (LS) in groups of sheep with known ovulation rates (ORs). However, some lines of sheep produce more lambs than would be predicted from their ORs alone [9,10], indicative of increased ES. The physiology that underlies this ability to produce more lambs is currently unknown.

Previous studies have shown that increases of progesterone advance embryonic development in sheep [11] and are associated with enhanced ES in both sheep [12] and cattle [13–16]. Although early bovine embryos have been shown to express progesterone receptors, Clement et al. [17] reported that the actions of early exposure to progesterone were mediated by the uterine endometrium rather than via a direct effect on the embryo itself. In this study, we examined extreme outlier ewes that consistently produced more or less lambs in their lifetime than would be predicted from their OR, with a focus on determining whether differences in progesterone concentrations accounted for observed differences for this trait. Specifically, progesterone concentrations in peripheral plasma during the estrous cycle and early gestation were compared. Additionally, ovarian and uterine vein and uterine luminal concentrations of progesterone were assessed during early gestation in these ewes. The ability of the uterus to synthesize progesterone *de novo* was also examined.

2. Materials and methods

2.1. Experimental design

All animal experiments were conducted following approval by the Animal Ethics Committee of the AgResearch Invermay Agricultural Centre, in accordance with the 1999 Animal Welfare Act (Codes of Ethical Conduct) of New Zealand.

2.2. Selection of ewes

ES was determined as the deviation of the mean observed LS from that predicted by the mean observed OR derived from the equation published by Hanrahan et al. [10]:

$$Predicted\ LS = 0.15 + (0.926 \times OR) - (0.0763 \times OR^2)$$

$$ES = Observed\ LS - Predicted\ LS$$

Multiparous Coopworth and Coopworth Texel cross ewes with a lifetime history (≥3 records) of enhanced (≥0.15) or reduced (≤−0.10) ES were selected from the AgResearch Woodlands Gene Flock.

2.3. Design of experiments

Four experiments were undertaken to examine the role of progesterone in early ES. In the first two experiments, daily concentrations in jugular venous plasma from 5 days before and up to 14 days after the day of estrus onset (Day 0) were measured in enhanced and reduced ES ewes. This was done during an estrous cycle where ewes were mated to a vasectomized ram (experiment 1; n = 5 enhanced ES ewes and n = 4 reduced ES ewes) and during early gestation after mating of ewes with a fertile ram (experiment 2; n = 13 enhanced ES ewes and n = 10 reduced ES ewes). In experiment 3, progesterone concentrations in plasma from the jugular vein, left and right ovarian, and uterine veins and uterine fluid were compared between the enhanced (n = 17) and reduced (n = 6) ES ewes at Day 5 of gestation, the period when the embryo is expected to enter the uterus from the oviduct. In experiment 4, the ability of the uterus to synthesize progesterone *de novo* in the enhanced (n = 4) and reduced (n = 4) ES ewes was examined by reverse transcriptase PCR.

2.4. Estrous synchronization

Ewes were synchronized with either two im injections of 0.7 mL of estrumate (active ingredient cloprostenol, a PGF2α analogue, Intervet, Upper Hutt, New Zealand) given 9 days apart in the middle of the breeding season (experiments 1 and 2), or an insertion of a progesterone controlled-internal-drug-release device (Southern Veterinary Supplies, Christchurch, New Zealand) for 13 days with a new controlled-internal-drug-release introduced at Day 10 (experiments 3 and 4) during the breeding season. The onset of estrus (Day 0) was detected by mating with a vasectomized or fertile ram. Live weights of all ewes were measured at the start of the experiment. All blood and tissue samples were collected during the second estrous cycle after synchronization, so that samples represent those of ewes during a natural breeding cycle.

2.5. Sampling

In experiments 1 and 2, 10 mL heparinized blood samples were collected daily from the jugular vein. For experiment 3, in addition to one jugular vein sample, 1 mL heparinized blood samples were taken from both the left and right ovarian and uterine veins under general anesthesia, which was induced by intravenous bolus of 5% thiopental sodium (Thiobarb, Southern Medical Products, Dunedin, New Zealand) and maintained by inhalation of halothane (Southern Veterinary Supplies) [18]. For