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Failure to establish and maintain a pregnancy in undernourished recipient ewes is associated with a poor endocrine milieu in the early luteal phase

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ABSTRACT

Embryos from undernourished and control donor ewes were transferred to undernourished and control recipient ewes. Progesterone and metabolic hormones were investigated in recipient ewes to determine their association with pregnancy success. Forty-five donor and 52 recipient Rasa Aragonesa ewes were fed 1.5 (control group; donor n = 20; recipient n = 25) or 0.5 (low group; donor n = 25; recipient n = 27) times the daily requirements for maintenance from the onset of estrous synchronization treatment to embryo collection and transfer. The embryos were collected 7 days after the onset of estrus (day 0), and two good-quality embryos were transferred into each recipient ewe. The percentage of pregnant ewes on day 18 and 40 did not differ between the two groups, although the recipient undernourished ewes tended to have greater late embryonic mortality (from days 18–40) than the control recipient ewes (P = 0.11). No effect of the nutrition of the donor was found. Recipients that became pregnant had a higher ovulation rate than non-pregnant ewes (P = 0.02). Undernourished ewes had lower plasma insulin concentrations than control ewes (P = 0.03), and those that suffered late embryo mortality (from days 18–40) tended to have lower insulin and progesterone concentrations than their counterparts that remained pregnant (P = 0.06 and P = 0.07, respectively). In this study, pregnancy in control and undernourished recipient ewes was not associated with the origin of the embryo (undernourished and control donors). In conclusion, failure to establish and maintain a pregnancy was associated with lower progesterone and insulin levels one week after estrus in recipient ewes.

1. Introduction

Nutritional status is one of the most important factors affecting reproduction in sheep; the physiological pathways by which the hypothalamic-pituitary-ovarian-uterine axis is informed about the energy status of the animal are complex and involve several metabolites and

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hormones (e.g., growth hormone (GH), insulin-like growth factor 1 (IGF1) system, insulin, leptin and adiponectin) (Blache et al., 2000). It has been demonstrated that the majority of the reproductive losses in ruminants (25–55%) occur during the early embryonic period (Niswender and Nett, 1994). The potential causes of wastage include abnormalities of the ovum or the embryo, luteal inadequacy and/or failure of the supply of progesterone to the uterus, and failure of the systems of maternal recognition of pregnancy (Rhind, 1992). The nutritional status of the mother, which may be acting before or after ovulation, may affect oocyte fertility, embryo development and pregnancy establishment, which may be acting before or after ovulation.

Ovulation has been reported by some researchers to alter oocyte morphology (Lozano et al., 2003; Yaakub et al., 1997), but some studies have reported no effect relative on a control group (Boland et al., 2001; Kakar et al., 2005). The fertility of oocytes collected from undernourished ewes was reported to be lower than that of ewes fed the maintenance diet (Lozano et al., 2003; O'Callaghan et al., 2000), and we have shown that a 0.5 maintenance diet offered to superovulated ewes resulted in a lower total number of embryos and number of viable-transferable embryos compared with control animals (Abecia et al., 2015). Rhind et al. (1989) demonstrated that a 25-day undernutrition treatment increased embryo mortality at day 11 of gestation; other studies with similar low nutrition treatments have yielded embryos with retarded development (Abecia et al., 1997, 1999; Lozano et al., 2003).

The relationship between nutritional level and fertility has been also evaluated after ovulation and during the early luteal phase. It has been postulated that undernutrition during the early luteal phase affects peripheral progesterone concentrations, which are necessary for adequate uterine preparation for embryo growth and pregnancy maintenance. Indeed, a negative association between the level of feed intake and circulating progesterone concentrations has been demonstrated, likely due to the lower clearance rate in the liver (Parr et al., 1987; Rhind et al., 1989). Lozano et al. (1998) also observed that higher plasma progesterone concentrations of undernourished ewes were associated with lower contents of progesterone in the uterus on day 5 of the estrous cycle. There were also a smaller number of progesterone receptors at this time (Sosa et al., 2004, 2006).

Although there is a wide body of work related to endocrine responses to a negative energy balance in ruminants (mainly insulin, IGF1, leptin and, to a lesser extent, adiponectin), only a few studies have focused on the association among endocrine profiles and reproductive success. Leptin has also been implicated in embryo development and implantation (Kawamura et al., 2002), but less research has been conducted on the adiponectin role in pregnancy.

The present study was performed to differentiate between the effects on embryo development of undernutrition from maternal effects. This study relied on a multiple ovulation and embryo transfer model; embryos from undernourished and control donor ewes were transferred to undernourished and control recipient ewes, and several reproductive outcomes (ovulation rate, pregnancy rate, early and late embryo losses) were assessed. Moreover, progesterone and metabolic hormones were investigated in recipient ewes in order to determine their association with pregnancy success.

2. Material and methods

The study was conducted at the experimental farm of the University of Zaragoza, Spain (latitude 41°N). All of the procedures were carried out under Project License PI05/10 approved by the in-house Ethics Committee for Animal Experiments from the University of Zaragoza. The animals were cared for and used in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 related to the protection of animals used for experimental and other scientific purposes.

2.1. Animals and treatments

The experiment was conducted in October during the breeding season using Rasa Aragonesa ewes with a mean (± Standard Error of the Mean, SEM) body weight (BW) of 57.1 ± 1.4 kg and an initial body condition score (BCS) above 3 (scale from 0 = thin to 5 = obese; Russel et al., 1969) that were housed in communal yards that had uncovered areas. The animals were offered a diet formulated to fulfill their maintenance requirements (Agricultural and Food Research Council (AFRC), 1993) one month prior to the onset of the experiment. The diet included 0.42 kg of pellets and 0.70 kg of barley straw per day, which provided 7.8 MJ of metabolizable energy per day per ewe. The pelleted diet consisted of barley (85%) and soybean (15%). The animals had unrestricted access to water and mineral supplements.

The ewes were synchronized in estrus (day 0) using intravaginal sponges that contained 30 mg fluorogestone acetate (Chronogest, MSD, Madrid, Spain), which were inserted for 14 days. Only animals that showed estrus were included in the study (n = 97). These animals were placed in a randomized block design with four temporal replications (n = 12 or 13 per treatment and replication). At the time of sponge insertion, the ewes were allocated to one of two groups to be fed diets that provided either 1.5 (control group; donor n = 20; recipient n = 25) or 0.5 (low group; donor n = 25; recipient n = 27) times the daily requirements for maintenance. During the experimental period, the diets of the ewes consisted of 0.55 or 0.1 kg of pellets and 0.8 or 0.5 kg of barley straw for the control and low groups, respectively. These regimens were maintained up to the day of embryo collection (day 7). Body weight and BCS on a scale of 0–5 (0 = emaciated and 5 = obese; Russel et al., 1969) were determined at time of sponge insertion (day −15), withdrawal (day −1) and at embryo transfer (day 7).

Superovulation of donor ewes (control group n = 20; low group n = 25) was induced with 210 mg of pFSH (Folltropin; Bioniche Animal Health, Belleville, ON, Canada) and 500 IU eCG (Folligon, MSD Salud Animal, Madrid, Spain) in a single im administration 48 h prior to the intravaginal sponge being removed. Seven days after the onset of estrus, embryos were collected through a mid-ventral laparotomy and classified based on their stage of development and morphology according to Winterberger-Torres and