Journal of Animal and Veterinary Advances 9 (11): 1621-1624, 2010
ISSN: 1680-5593
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Effects of Energy Level and PMSG Dose on Blood Progesterone, Insulin and FSH Concentration in Zel Ewes Prior to and after Mating

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Abstract: About 184 Zel ewes, 3-5 years of age and a body weight of between 40 and 45 kg were used in the trial. Ewes were randomly allocated to 4 treatments groups based on BW and age (46 ewes/group). All of the ewes were fed in two nutritional groups including low (2 mcal kg^{-1}) and high (2.3 mcal kg^{-1}) metabolizeble energy diet. Ewes received experimental diet until 28th day of experiment. The estrous cycles of ewes were synchronized using SIDR and 2 levels of PMSG (300 and 500 IU). Treatments include: 1-High energy and 300 IU PMSG (H300), 2-High energy and 500 IU PMSG (H500), 3-Low energy and 300 IU PMSG (L300) and 4-Low energy and 500 IU PMSG (L300). Jugular blood samples were collected from ewes using vacutainers at 10 h in first day of experiment, CIDR insert day, CIDR removal day before mating and 120 h after mating. Bloods samples centrifuged at 3000× g for 15 min then serum immediately separated and kept frozen at -20°C until analysis for insulin, FSH and progesterone. Repeated measurements used for data analysis. The result showed that there were no any significant difference between two groups weight before start the experiment ($p>0.05$). During the experiment high level of energy increased the body weight than low level group ($p<0.05$). Energy had no significant effect on blood FSH and progesterone concentration ($p>0.05$) but high level of energy decreased the insulin concentration significantly ($p<0.05$). In this study PMSG had no any significant effect on blood metabolites such as FSH, Insulin and progesterone.

Key words: Progesterone, ewe, blood, zel, PMSG, centrifuged

INTRODUCTION

Among the environmental factors influencing reproduction in sheep, the level of nutrition is one of the most important. The interaction between nutrition and reproduction has major implications for the reproductive performance of sheep and this has been known for some time. Over the years, a considerable body of research has identified three recognized and generally accepted effects of nutrition on ovulation rate: the static effect, the dynamic effect and the immediate effect (Smith and Stewart, 1990). Insulin is a key hormone controlling glucose metabolism because it increases the ability of the cells to take up glucose and an increase in blood glucose signals the release of insulin from ß cells of the pancreatic islets. Insulin has specific effects on granulosa and theca cell function. The insulin-dependent glucose transporter (GLUT-4) is present in granulosa and theca cells of sheep follicles; the implication being that changes in insulin mediated glucose uptake within the ovary modulates follicular function. Insulin is an important regulator of folliculogenesis because of either its general regulation of cellular glucose uptake or its direct action on folliculogenesis either way suggesting a role for insulin in the mechanism of nutritional effects on folliculogenesis in sheep (Downing *et al*., 1995). Estrous synchronization allows lamb producers to maximize facilities and labor as well as yield a more uniform lamb crop at optimal times throughout the year. Progesterone is central to the complex regulation of normal reproductive function and both duration and level of progesterone pre-exposure have a major effect on the estrogen-dependent LH surge (Skinner *et al*., 2002). Controlled Internal Drug Releasing Devices (CIDR) are an intravaginal progesterone delivery method and are constructed of a progesterone impregnated medical silicone elastomer molded over a nylon core (Wheaton *et al*., 1993).

Hamra *et al*. (1986) reported that progesterone concentrations in ewes treated with CIDR devices increased to near maximum values within 24 h, peaked at 4 days after insertion and decreased there after. These CIDR inserts have been shown to be highly efficient

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synchronizing agents in ewes (Greyling and Brink, 1987) and produce greater serum progesterone concentrations than polyurethane sponges (Hamra *et al*., 1986). The aim of this study was to investigate the effects of energy level and PMSG dose on blood progesterone, insulin and FSH concentration in ewes prior to and after mating.

MATERIALS AND METHODS

Study site: The experiment was conducted between 11 August and 13 September 2007 at the research farm of Jihad-Agriculture Ministry in Golestan Province located 30 km from Gorgan in Iran. The yearly absolute minimum and maximum temperatures in the area are on average 0 and 40°C centigrade, respectively whereas the mean annual rainfall is 560 mm.

Experimental animals and their management: A total of 184 Zel ewes, 3-5 years of age and a body weight of between 40 and 45 kg were used in the trial. Zel is only breed in Iran that doesn't have fat tail. Ewes were randomly allocated to four treatments groups based on BW and age (46 ewes/group). Animals were housed in pens and allowed free access to water.

Treatments and procedures: All of the ewes were fed in two nutritional groups including low (2 mcal kg^{-1}) and high (2.3 mcal kg^{-1}) metabolizeble energy diet. Ewes received experimental diet until 28th day of experiment. The estrous cycles of ewes were synchronized using SIDR (natural progesterone) and two levels of PMSG (pregnant mare serum gonadotrophin) (300 and 500 IU). CIDR (CIDR, Inter Ag, New Zealand) insert in day 12 of experiment and removed after 14 days and PMSG (300 and 500 IU, Pregnecol, Injection, Australia) injected at mentioned dose simultaneously. Treatments Include: 1-High energy and 300 IU PMSG (H300), 2-High energy and 500 IU PMSG (H500), 3-Low energy and 300 IU PMSG (L300) and 4-Low energy and 500 IU PMSG (L300). Rams were introduced to groups 36 h after PMSG injection. During the experiment ewes were fed according to National Research Council (1985) nutrient requirement. Animals received the diet in 2 times day^{-1} (morning and evening).

Blood sampling and analysis: Jugular blood samples (10 mL) were collected from ewes using vacutainers at 10 h in 1st day of experiment, CIDR insert day CIDR removal day before mating and 120 h after mating. Bloods samples centrifuged at 3000 g for 15 min, then serum immediately separated and kept frozen at -20°C until analysis for insulin, FSH and progesterone.

Statistical methods: A completely randomized design in factorial arrangement with two factors (energy and PMSG) and two levels were used. The data were checked for errors and compared with written reports; outliers were rechecked to ensure that values were accurate. All outcome variables were screened for normality by visual assessment of the distributions and calculation of kurtosis and skewness. Repeated measurements analysis for blood samples used the MIXED procedure of SAS (1999). The level of significance was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effects on body weight: Before the experiment all of the ewes weighted and divided into two groups. There were no any significant difference between two groups weight before start the experiment ($p>0.05$). During the experiment high level of energy increased the body weight than low level group ($p<0.05$) (Table 1). High-energy diets have been shown to improve growth rates in different sheep breeds. Beauchemin *et al*. (1995) studied the effect of dietary energy density on growth performance of Rambouiller, Dorset, Finn, Sufflok and Ramanov breeds and observe that reducing the dietary digestible energy reduce growth rate and decrease growth efficiency. Mahgoub *et al*. (2000) reported similar results with Omani lambs. Differences between sheep breeds in their ability to ingest, digest, adapt and respond to dietary treatments have been reported by Lourenco *et al*. (2000), Givens and Moss (1994) and Ranilla *et al*. (1997). Weyreter and Engelhardt (1984) observe that Heidschucken sheep adapted better to low quality forage diets compared with Merino sheep while Blackhead sheep were not able to make such an adaptation. Additionally, Karim and Santra (2000) concluded that feed efficiency for Malpura lambs is better than random bred Malpura lambs.

Effects of energy level on blood metabolites: Energy had no significant effect on blood FSH and progesterone concentration ($p>0.05$) but high level of energy decreased the insulin concentration significantly ($p<0.05$) (Table 2).

Table 1: Effects of energy level on ewe weight

	Energy level		
Item	2.3 mcal kg^{-1}	2 mcal kg^{-1}	SE
Ewe weight before experiment	36.63[a]	35.16[a]	1.15
Ewe weight after experiment	40.14[a]	35.61[b]	1.25

Table 2: Effects of energy level on blood FSH, insulin and progesterone

	Energy level		
Item	2.3 mcal kg^{-1}	2 mcal kg^{-1}	SE
FSH	0.4876[a]	0.4888[a]	0.00030
Insulin	22.7324[b]	24.6591[a]	0.56920
Progesterone	1.1598[a]	1.1073[a]	0.03401

Table 3: Effects of PMSG level on blood FSH, insulin and progesterone

	PMSG		
Item	300 IU	500 IU	SE
FSH	0.4916[a]	0.4926[a]	0.00160
Insulin	24.0907[a]	23.3008[a]	0.57270
Progesterone	1.1299[a]	1.1372[a]	0.03406

Table 4: Effects of treatments on blood FSH, insulin and progesterone

	Treatments				
Item	H300	H500	L300	L500	SE
FSH	0.4917[b]	0.4933[a]	0.4924[ab]	0.4928[ab]	0.0024
Insulin	22.8154[b]	22.6494[b]	25.3659[a]	23.9522[ab]	0.8055
Progesterone	1.1398[a]	1.1798[a]	1.1200[a]	1.0946[a]	0.0481

Increased protein or energy intakes result in higher ovulation rate and circulating concentrations of insulin (Smith and Stewart, 1990). Insulin infusion has been shown to increase ovulation rate by Hinch and Roelofs as reported by Smith and Stewart (1990) but not by Beam and Holcombe (1992). However, high insulin levels may result from insulin resistance known to affect ovulation rate negatively in Boorola crossbred ewes (Landau, 1993). Results of the present study suggested that insulin concentration decreased by high amount of energy.

In cattle, Hill *et al.* (1970) found a decrease in plasma progesterone level on submaintenance (85%) energy and protein with no change in Luteinizing Hormone (LH). Plasma progesterone level in heifers and cows on restricted energy intake was higher than in non-restricted animals during the first cycle but the level became progressively lower in the subsequent two cycles (Donaldson *et al.*, 1970; Gombes and Hansel, 1973). It has been reported that under nutrition increased plasma progesterone concentrations in pregnant cows in mid and late pregnancy. There are considerable inconsistencies regarding the effect of nutrition on plasma progesterone concentrations during the reproductive cycle (Beal *et al.*, 1978).

Effects of PMSG level on blood metabolites: In this study PMSG had no any significant effect on blood metabolites such as FSH, Insulin and progesterone (Table 3). The effect of treatments is shown in Table 4. Use of progesterone concentration after superovulation as a prognostic tool for the prediction of ovulation rate and/or embryo yield has been met with limited success. Although, many researchers have found a relationship between ovarian response (number of CL) and blood progesterone levels after superovulation, the success in predicting the exact number of ovulations, eggs or viable embryos is limited (Bindon *et al.*, 1971; Booth *et al.*, 1975; Greve *et al.*, 1983; Saumande *et al.*, 1985). It has also been reported that plasma concentration of progesterone at the beginning of oestrus is inversely related to embryo quality in cattle (Jensen *et al.*, 1982; Goto *et al.*, 1987; Britt and Holt, 1988). Buserelin injection during the mid luteal phase has been reported to increase plasma LH and progesterone concentrations and cycle length in cattle and cycle length in sheep (Macmillan and Sealey, 1989). Furthermore, it has been shown that GnRH treatment stimulates the release of pituitary gonadotrophins that improve luteal function and luteinise developing follicles. The net effect is a transitory increase in plasma progesterone and oestradiol concentrations followed by a prolonged decrease in oestradiol concentrations. It is assumed that this observed decrease in oestradiol concentrations as a result of buserelin (GnRH) treatment delays luteolysis, as higher oestradiol concentrations are required to induce an increase in oxytocin receptor concentrations (Macmillan and Sealey, 1989).

CONCLUSION

During the experiment high level of energy increased the body weight than low level group. Energy had no significant effect on blood FSH and progesterone concentration but high level of energy decreased the insulin concentration significantly. In this study PMSG had no any significant effect on blood metabolites such as FSH, Insulin and progesterone.

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