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Effect of transient high-energy diets just after ovulation on ovarian performance and metabolic status in cyclic ewes

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ABSTRACT

The aim of this study was to investigate the influence of short-term treatment of high-energy diet after ovulation on ovarian function and metabolic status in ewe. Cycling ewes Ossimi were divided into treatment group (TG; $n=6$) and control group (CG; $n=6$). After ultrasonographic detection of ovulation (day 0), a high-energy diet (12.55 MJ DE/kg diet; 125% of maintenance) was fed to TG from day 1 to day 4 after ovulation (4 days). The high-energy diet consisted of 850 g concentrate mixture and 150 g alfalfa hay, plus *ad libitum* access to wheat straw. The CG was offered as maintenance diet (10 MJ DE/kg diet) throughout the experiment. Follicular development was observed ultrasonographically every other day while blood samples were collected daily throughout the experiment for the analysis of glucose, total cholesterol, urea, triglycerides, total proteins, aspartate aminotransferase (AST), alanine transaminase (ALT) and glutamate oxaloacetate transaminase (GOT). Transient feeding of high-energy ration during early luteal phase of estrous cycle significantly influenced the concentration of glucose and some metabolic profiles. Total proteins were greater at first, third and fourth day after ovulation (ovulation is day 0) in TG when compared to CG ewes. Blood glucose concentration was greater in TG than that of CG at days 1, 2, 3 and 4 postovulation. The average number of small follicles (2–2.9 mm) of the first follicular wave after ovulation was greater in TG ewes (23.3 follicles) than that of CG (2.2). There was no difference in the number of medium (3–5 mm) and large sized follicles (>5 mm) between CG and TG ewes. Moreover, the maximum size of large follicles did not differ between CG (5.6 mm) and TG ewes (5.9 mm). In conclusion, high-energy diet may improve number of small follicles and alter energy metabolite during early luteal phase in cycling ewes.

1. Introduction

The effects of nutrition on reproduction are well known and widely reported (Scaramuzzi et al., 2006). The reason for this marked association between nutrition and reproduction is to ensure that reproduction is very closely aligned with the food supply.

Energy balance shows the relationship between nutrition and reproduction (Scaramuzzi et al., 2006). When the net nutrient requirement is equal to net nutrient intake, the animal is in a state of energy balance. Positive energy balance leads to increased leptin and insulin concentrations in the blood and increased glucose uptake; these changes appear to affect the ovary directly and are associated with increased folliculogenesis and increased ovulation rate in sheep (Downing et al., 1995; Munoz-Gutierrez et al., 2002).

The manipulation of reproduction using nutrition is an inexpensive management tool to control ovulation rate and litter size particularly in low cost, extensive

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production systems in marginal environments such as the semi-arid, Mediterranean and hill farming regions of the world (Martin et al., 2004). A more complete understanding of how and when nutrition affects ovulation rate will facilitate the application of targeted nutrition in sheep production systems to optimize reproduction and to provide an alternative approach to managing reproduction in commercial systems that do not depend on the use of exogenous hormones. High energy and high protein supplement is sufficient to increase the ovulation rate in sheep (Gherardi and Lindsay, 1982; Oldham and Lindsay, 1984; Stewart and Oldham, 1986). Short-term supplements have to be fed during the time the ovulatory wave emerges (Nottle et al., 1985, 1990; Stewart and Oldham, 1986; Downing et al., 1995).

Gonadotrophins are possibly not involved in the initiation of follicular development. Furthermore, at the early stages of follicular growth, gonadotrophins appear not to be a definite requirement for follicular development (Campbell et al., 2003). However, at the later stages follicular growth is clearly dependent on the pituitary gonadotrophins, LH and FSH. These hormones provide the primary mechanisms that control follicular dynamics including recruitment, selection and dominance *via* negative inhibitory feedback loops with the hypothalamo-pituitary unit.

The aim of the present study was to establish a novel method of short-term nutritional supplementation that could stimulate ovarian function in ewes during early luteal phase post-ovulation.

2. Materials and methods

2.1. Animals

The experiment was carried out at the experimental farm of faculty of agriculture, Alazhar University, Assiut (27°N, 31°E), Egypt. Multiparous Ossimi ewes (age = 3–4 years) were fed a maintenance diet of 600 g alfalfa hay, 100 g wheat straw and 300 g of concentrate mixture (10 MJ DE/kg diet). Ossimi ewes are seasonally polyestrous animals during autumn (September and October).

2.2. Experimental procedure

The estrous cycles of ewes were synchronized by intramuscular double injection of 10 mg of Dinoprost (Lutalyse, Pharmacia, Belgium), and ovulation was confirmed by ultrasonography. They were randomly assigned into control group ($n = 6$, 45–50 kg body weight; CG) and treatment group ($n = 6$, 45–50 kg body weight; TG). The animals were examined by ultrasonography and introduction of ram once daily in order to estimate the day of estrus and subsequent ovulation. After detection of ovulation (day 0), the treatment group was fed a high-energy diet from day 1 to day 4 after ovulation (for 4 days). The composition of high-energy diet (12.55 MJ DE/kg diet; 125% of maintenance) was 850 g concentrate mixture and 150 g alfalfa hay, plus *ad libitum* access to wheat straw (Table 1).

Treatment group received maintenance ration with the exception of these days while control group received maintenance ration throughout the experimental period.

2.3. Ultrasonographic examination and blood sampling

The ovaries were examined *via* transrectal ultrasonography, using a real-time, B-mode, diagnostic scanner equipped with a transrectal 6/8 MHz linear array transducer (Pie Medical, 100 LC, Holland). Follicular dynamics were estimated every other day by ultrasonography from the first ovulation to the second ovulation. In combination with ultrasonography, estrus of the animals was detected by checking behavior (refusal or standing) after introducing a ram to females once daily.

Table 1
Components and calculated composition of experimental diets.

Ingredient, %	Maintenance	High energy
Alfalfa hay	60	15
Wheat straw	10	*Ad libitum*
Concentrate mixture	30	85
Calculated nutrient (%)		
DE Mcal/kg	2.4	3.00
DE MJ/kg	10.00	12.55
CP	10.6	11
CF	29.8	6.68
EE	4.01	7.6
NFE	40.86	62.29
Ash	5.74	4.94
OM	85.26	87.64
DM	91	92.58
Ca	0.76	0.55
P	0.31	0.36

Concentrate mixture: consisted of 80% yellow corn, 13% wheat bran, 3% undecorticated CSC, 2% soybean meal (42%), 1.2 limestone, 0.3 vitamin and Mineral premix, 0.5% common salt.
Trace element and vitamin premix each 3 kg contain 1,250,000 IU Vit. A; 2,500,000 IU Vit. D3; 1000 mg Vit E; 80,000 mg Mn; 60,000 mg Zn; 50,000 iron, 20,000 copper, 5000 iodine, 250 Se, 1000 Co mg tell 3 kg $CaCO_3$.

The term wave was defined as one or more antral follicles growing from 2 to ≥5 mm in diameter before regression. Ovarian follicles ≥2 mm in diameter were measured and their relative locations were noted on an ovarian map to follow the sequential follicular development, and the mean day of emergence of the ovulatory follicles, the mean number of small (2–2.9 mm in diameter), medium (3–5 mm in diameter), and large follicles (>5 mm in diameter) were recorded. The first ultrasonic detection of ovulatory follicles of 2 mm in diameter was considered as the day of emergence. When this follicular diameter was more than 2 mm at first detection, the previous day was considered as the day of emergence. The day of ovulation was regarded as the day on which a large growing follicle that had been identified and followed for several days was no longer seen.

Blood samples were collected by jugular venipuncture daily until 6 days after second ovulation. Blood samples were centrifuged at 3000 rpm for 20 min and serum was harvested and stored at −20 °C until assayed for glucose, total cholesterol, urea, triglycerides, total proteins, aspartate aminotransferase (AST), alanine transaminase (ALT) and glutamate oxaloacetate transaminase (GOT).

2.4. Analysis of blood metabolites

Blood metabolites were analyzed by spectrophotometer (Unico, USA) using commercial test kits (Spectrum Company, Egypt): glucose – enzymatic colorimetric method (Weissmann and Klein, 1958), total protein – Biuret reagent (Gornall et al., 1949), albumin – bromocresol green reaction (Doumas et al., 1971), total cholesterol (Ellefson and Caraway, 1979), urea (Tietz, 1990), serum triglycerides (Bucolo and David, 1973) and AST and ALT (Breuer, 1996).

2.5. Statistical analysis

An analysis of variance was used for the comparison between treatment groups regarding the mean number of follicles, mean day of emergence and maximum size of the ovulatory follicles. The data were subjected to statistical analysis with independent *t*-test of (SPSS for Windows Version 16; SPSS GmbH, Munich, Germany). Probability values of less than 0.05 ($P < 0.05$) were considered significant. Results are expressed as means ± SEM.

3. Results

3.1. Glucose and some metabolic profiles

The mean concentrations of serum total protein increased significantly at the first (7.9 ± 0.3 g/dl;