*Full Length Research Paper*

# Impact of a short period-energy supplementation on the ovarian follicular dynamics, blood metabolites and sex hormones in ewes

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The effect of energy supplementation for a short period on follicuar turnover and estrogen concentration during the estrus cycle in subtorpics was studied in 13 ewes (7 ewes subjected to high energy, HEG and 6 as normal energy group, NEG). After ovulation (Day 0), a high-energy diet (10.87MJ ME/kg diet; 130 % of maintenance) was fed to HEG from Day 1 to Day 4 after ovulation and from Day 8 to Day 11 of the cycle (4 days each). The high-energy diet consisted of 850 g concentrate mixture and 150 g alfalfa hay, plus ad libitum access to wheat straw. The NEG was offered as maintenance 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 albumin, globulin, glucose, total cholesterol, urea, triglycerides, total proteins, estrogen and progesterone. Transient feeding of high-energy ration during early and mid luteal phase of estrous cycle significantly influenced the concentration of glucose and some metabolic profiles. A marked rise in the concentrations of glucose appeared in response to the intermittent nutritional stimulus. Mean plasma concentrations of glucose were significantly ($P < 0.05$) higher in the HEG than in the NEG on almost all days during treatment period. For ovarian performance, the total number of medium and large follicles that developed on the day prior to the 2nd ovulation in the HEG ($3.5\pm0.17$) was significantly higher than that of the NEG ($1.87\pm0.26$). Ovulation rate ($2.8\pm1.0$) in the HEG was significantly higher than that of the NEG ($1.15\pm0.6$). No significant difference was detected in the concentrations of progesterone and estrogen throughout the experiment between groups except for E2 peak concentration on Day 8. The present experiment demonstrated that short-term intermittent nutritional stimulus in cyclic ewes increased the total number of ovulatory follicles and the ovulation rate in association with increasing plasma concentrations of glucose and peak levels of estrogen.

**Key words:** Ewe, follicular dynamics, transient energy, P4, E2, blood metabolites.

## INTRODUCTION

The nutritional effect on ovarian function was mainly explained by short-term influence of energetic status on the ovary and indirect action through the endocrine system (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. Nutritional supplementation for 7 days from the luteal phase to the follicular phase stimulates the pulse frequency of luteinizing hormone (LH) secretion and wave-like secretion of follicle stimulating hormone

(FSH) accompanied by increases in the plasma concentrations of glucose and insulin in cycling goats (Haruna et al., 2009). In cycling ewes, it was also demonstrated that the concentrations of glucose increased and remained at high levels for several days

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after the start of treatment, and then the levels decreased gradually to the baseline during the treatment period (Zabuli et al., 2009).

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

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 test the effect of short-term nutritional supplementation during the early luteal phase of the estrus cycle of the subtropical ewes on follicular dynamics and metabolite concentration.

## MATERIALS AND METHODS

### Animals

The experiment was carried out at the experimental farm of Faculty of Agriculture, Assiut University, Assiut (27◦N, 31◦E), Egypt. Thirteen multiparous Ossimi ewes (age = 3-6 years and 30-40 kg body weight) raised in a semi-open pens 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). The experiment was carried out during the winter breeding season for these animals from December to February.

### Experimental design

The estrous cycles of ewes were synchronized by intramuscular double injections (11 days apart) of 10 mg of Dinoprost (Lutalyse, Pharmacia, Belgium), and ovulation was confirmed by ultrasonography. They were randomly assigned into normal energy group (n = 6; NEG) and high energy group (n = 7; HEG). Estrus was detected using a teaser ram and ultrasonography was carried out daily to monitor ovulation. After detection of ovulation (Day 0), HEG was fed a high-energy diet from

Day 8 to Day 11 after ovulation (for 4 days) and Day 14 to Day 17 (for 4 days). The ingredients and chemical composition of experimental diets are shown in Table 1. Diets were mixed daily and fed twice a day and all nutrients met or exceeded the requirements of maintenance ewe sheep (NRC, 1985). The composition of high-energy diet (10.87MJ ME/kg diet; 130 % of maintenance) was concentrate mixture and plus ad libitum access to wheat straw. Feed intake was recorded daily and their representative samples were taken for chemical analysis. The average daily feed intake was 1231.43 ± 39.07 g/head/day and 1234.28 ± 22.58 g/head/day as fed for control and treated groups, respectively. HEG ewes received maintenance ration except during the two 4-days experimental periods, while NEG ewes group received a maintenance ration throughout the experimental period.

### Ultrasonographic examination and blood sampling

Ovarian structures of all animals were monitored ultrasonographically using a real-time, B-mode, diagnostic scanner equipped with a transrectal 5/7.5 MHz linear array transducer (Hitachi, EUB-405B, Japan). Ultrasound examinations were performed once daily from days 0 and each 12 h thereafter until ovulation or for a maximum of 48 h. All follicles >3 mm and CL were measured, and mapped individually for each ewe. Ovulation was considered to have occurred when a large growing antral follicle that had been identified and followed for several days then disappeared. Emergence of a follicular wave was defined as occurring on the day on which the retrospectively identified dominant follicle was 4 mm. The CL was examined and an image of the largest cross-sectional area was estimated. The following ovarian characteristics were determined and compared between groups: (1) ovulation rates; (2) diameter of the ovulatory follicles; (3) interval from treatment to emergence of a new follicular wave; (4) number and diameter of the CL.

Estrus was detected by checking behavior (refusal or standing) after introducing a ram to females thrice daily. The mean number of small (2 to 2.9 mm in diameter), medium (3 to 5 mm in diameter), and large follicles (>5 mm in diameter) were recorded. Blood samples were collected by jugular venipuncture daily until 6 days after second ovulation (23 days from the start of sponge withdrawal). Daily blood samples were centrifuged at 3000 rpm for 20 min and serum was harvested and stored at −20°C until assayed for total proteins, total cholesterol, globulin, glucose, urea, albumin, estrogen and progesterone.

### Analysis of blood metabolites and hormones

Blood metabolites were analyzed by spectrophotometer (Unico, USA) using commercial test kits (Spectrum

**Table 1.** Ingredients and chemical composition of experimental diets.

| Ingredient, % (as fed) | Maintenance | High Energy |
|---|---|---|
| Yellow corn | 20 | 85 |
| CSC, undecorticated | | 13 |
| Wheat bran | 53.2 | |
| Premix* | 0.30 | 0.30 |
| Ground lime stone | 1.00 | 1.00 |
| C salt | 0.5 | 0.5 |
| Wheat straw | 25 | *Ad libitum* |
| Total | 100.00 | 100.00 |
| | | |
| **Calculated nutrient (%)** | | |
| ME Mcal/kg | 2.00 | 2.6 |
| ME MJ/kg | 8.4 | 10.87 |
| CP | 10.60 | 10.53 |
| CF | 16.69 | 5.09 |
| EE | 2.68 | 2.98 |
| NFE | 51.12 | 64.87 |
| Ash | 7.51 | 3.76 |
| OM | 81.09 | 83.47 |
| DM | 88.60 | 87.23 |
| Ca | 0.43 | 0.40 |
| P | 0.6 | 0.33 |

*CSC = Cold Stone Creamery
High energy (30% more than maintenance ration)
**Trace element and vitamin premix for each package of 3 kg contain 1250000 IU, Vit. A; 2500000 IU, Vit. D3; 1000, mg Vit E; 80000 mg Mn; 60000 mg Zn; 50000 mg? iron, 20000 mg? copper, 5000 mg? iodine, 250  mg? se, 1000 mg? Co mg and CaCO3 up to 3000g.

Company, Egypt): glucose by the enzymatic colorimetric method (Weissmann and Klein, 1958), total protein by Biuret reagent (Gornall et al., 1949), globulin and albumin by bromocresol green reaction (Doumas et al., 1971), total cholesterol (Ellefson and Caraway, 1979), and urea (Tietz, 1990). Estradiol 17β  and P4 concentrations were determined using direct ELISA technique. Kits were provided by Diagnostic System Laboratory Co. (DSL, Catalogue No. 3900, USA). The intra- and inter-assay coefficient of variations were 4.8 and 9.2% for estrogen and 3.6 and 12.43% for progesterone, respectively. The sensitivity of the assay was 2 pg for E2 and 0.12 ng for P4.

**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 (2011). Probability values of less than 0.05 (P < 0.05) were considered significant. Results are expressed as means ± SEM.

**RESULTS**

**Glucose and some metabolic profiles**

There was a marked increase in the concentration of plasma glucose in the HEG compared with the NEG (p < 0.005) on Day 4 of the cycle and a significant rise in total cholesterol and urea on Days 0, 1 and 3 (P< 0.05). There were non significant increases in serum concentrations of other metabolites in HEG compared with NEG during the studied days of the cycle (Figure 1).

**Follicular dynamics**

The average number of small follicles of the first follicular wave after ovulation did not differ significantly between groups. However, there was a significant increase in the number of medium (3 to 5 mm) and large sized follicles (>5 mm) in the HEG (P<0.05). The total number of medium and large follicles that developed on the day prior to the 2nd ovulation in the HEG (3.5±0.17) was significantly higher than that of the NEG (1.87±0.26). Ovulation rate (2.8±1.0) in the HEG was significantly higher than that of the NEG (1.15±0.6). Moreover, the maximum size of large follicles was greater in HEG (5.8 ±
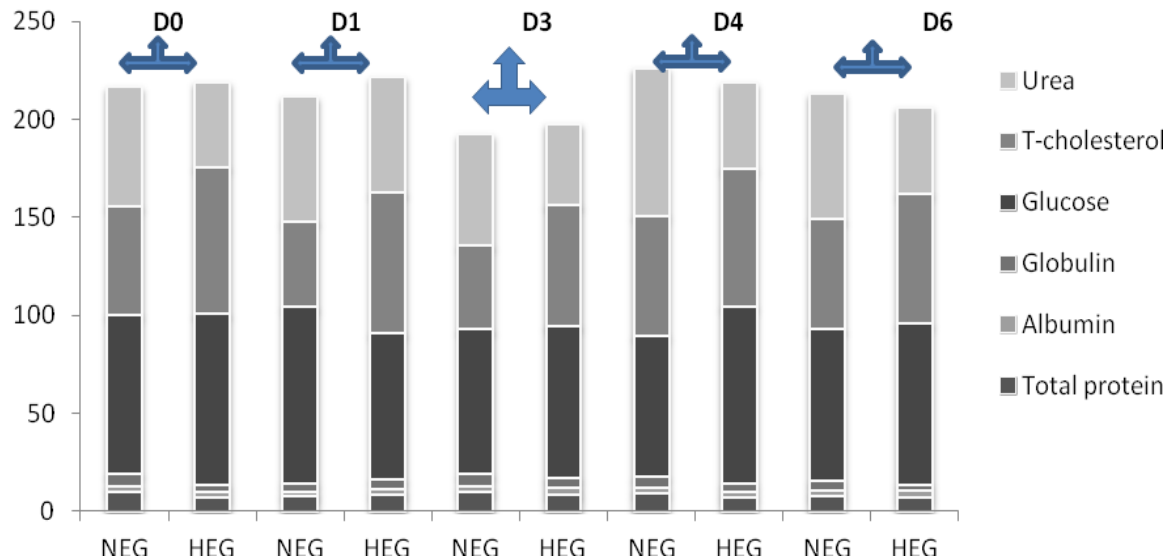
**Figure 1.** Changes in serum total protein (g/dl), total cholesterol (mg/dl), glucose (mmol/l), albumin (g/l), globulin (g/l), urea (mmol/l) during the early luteal phase of the estrus cycle in subtropical ewes treated with short term energy supplementation compared with control group (mean ± SE).
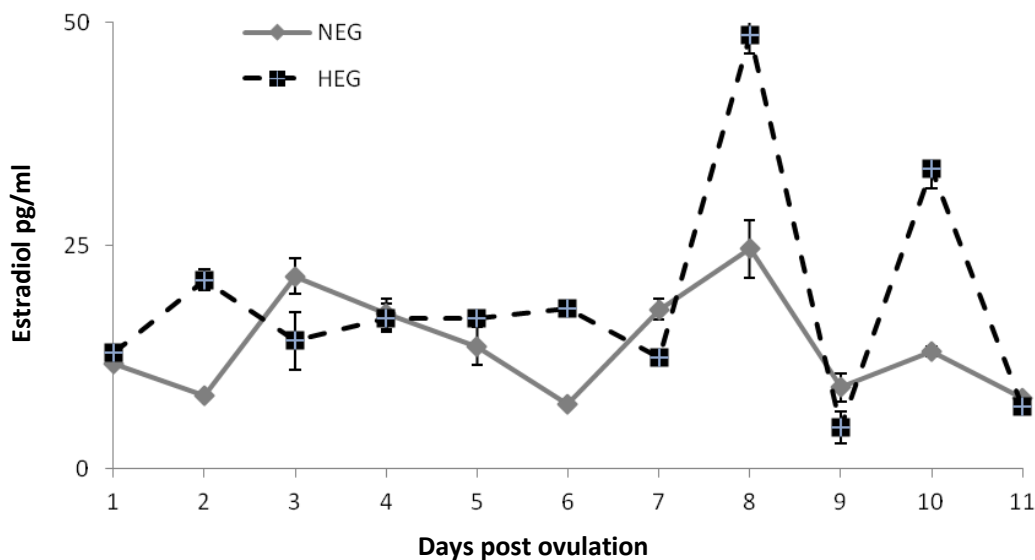


**Figure 2.** Changes in serum estrogen concentrations in HEG and NEG after ovulation in ewes flushed with short-termed energy supplementation (mean ± SE).

0.08 mm) compared with NEG (5.03 ± 0.01 mm) ewes. Out of the 6 ewes studied in the NEG, one ewe failed to ovulate.

**Serum E2 and P4 concentrations**

Serum E2 and P4 concentrations exhibited significant differences (P<0.05) between the studied groups but the HEG showed a significant increase in the peak level of estrogen on Day 8 post ovulation concomitant with presence of the second dominant follicle of the cycle in most studied animals within this group (Figure 2). Progesterone did differ significantly between the two groups (Figure 3).

**DISCUSSION**

The present study demonstrated that short-term
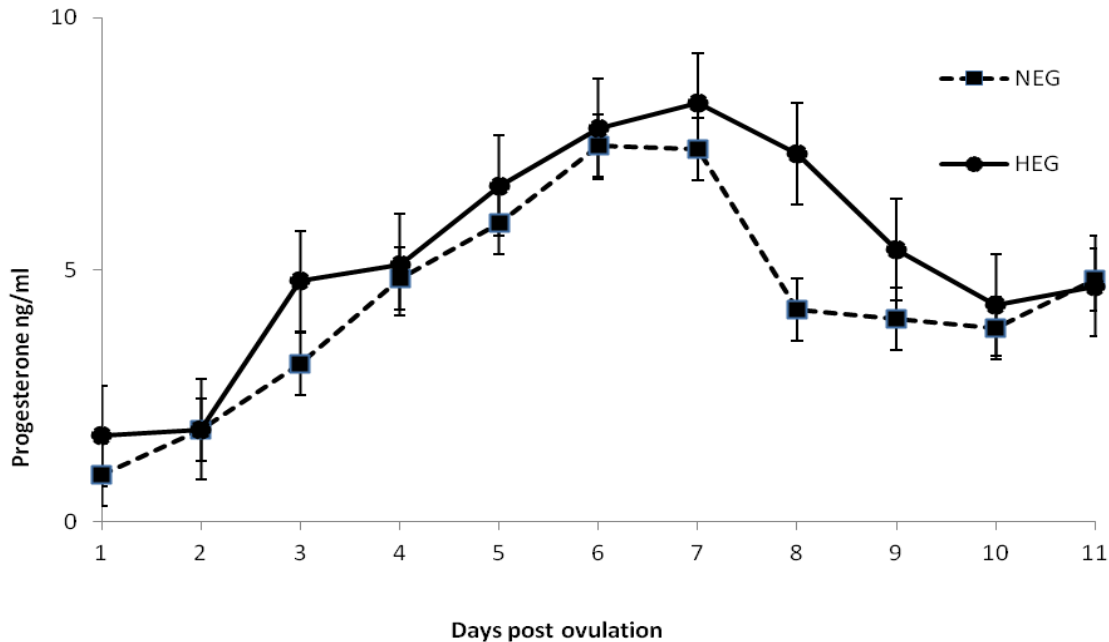
**Figure 3.** Changes in serum progesterone concentrations in HEG and NEG after ovulation in ewes flushed with short-termed energy supplementation (mean ± SE).

intermittent nutritional supplementation successfully increased the number of dominant follicles prior to ovulation, size of the ovulatory follicle and the ovulation rate. The significant improvement in blood metabolites during the transient high energy period could be attributed to the positive energy balance (Scaramuzzi et al., 2006). Nutritional flushing was found to alter the blood concentrations of some reproductive hormones using the short-term model: a transient increase in FSH and decrease in estradiol concentrations in the blood (Scaramuzzi et al., 2006).

In the ovary, the effect of nutrition is to stimulate folliculogenesis (Munoz-Gutierrez et al., 2002). The consequence of these direct actions on the follicle is a reduced negative feedback on the hypothalamic-pituitary axis which increases FSH secretion that leads to a stimulation of follicle maturation and transition to a larger follicular category. FSH is known to stimulate the final maturation of the dominant follicle together with LH. The metabolic responses to short-term high levels of feeding in the present study are in part similar to those of previous findings that blood concentrations of glucose and total cholesterol increased after the provision of supplemental feed comprising high digestible energy (Teleni et al., 1989; Vinoles et al., 2005; Haruna et al., 2009). However, several studies demonstrated that the concentrations of glucose and metabolic hormones reached peak values 2 or 3 days after the start of a high level of feeding, then decreased while the nutritional supplementation continued for 6 or 7 days in ewes (Vinoles et al., 2005) and in goats (Haruna et al., 2009).

The supplement increased the number of medium and large follicles. However, small follicles which are not dependant on gonadotrophins were not influenced by the level of nutrition. These findings were in contradiction to that of Vinoles et al. (2005). Although the exact mechanism controlling the effects of nutritional supplementation on folliculogenesis and the growth of dominant follicles is unclear, the present findings clearly indicate that increases in the number of ovulatory follicles were not due to changes in blood levels of progesterone and/or estradiol. Several lines of evidence suggest that increases in the blood glucose and insulin levels regulate glucose availability at the follicular level and folliculogenesis in ewes (Munoz-Gutiérrez et al., 2002; Letelier et al., 2008).

A recent review suggested that an increase in insulin-mediated glucose uptake by follicular cells may be critical for the growth of follicles and the prevention of atresia, thereby increasing the pool of ovulatory follicles (Scaramuzzi et al., 2006) supporting our findings. Therefore, one reason for the stimulatory effects on the number of the ovulatory follicles is likely to be that the first 4 days of nutritional supplementation mainly influences the follicular emergence of ovulatory follicles by modulating the intra-follicular insulin/glucose levels rather than the endocrine action (Zabuli et al 2010). Short-term nutritional supplementation also stimulates the frequency of pulsatile LH secretion during the follicular phase in goats (Haruna et al., 2009), and that its stimulatory effect is independent of body energy status of ovariectomized goats (Zabuli et al., 2009). In both

studies, a rise in LH pulse frequency was associated with increases in blood levels of glucose and insulin after nutritional supplementation and produced positive effects on the ovulatory follicles by during the follicular phase for the second ovulation. Therefore, it is possible that the first 4 days of nutritional supplementation made the ovary ready to respond to the stimulation of the second 4 days of nutritional supplementation and these actions increase the number of ovulatory follicles and ovulation rates. Supplementary feeding for several weeks is consistently associated with an increase in ovulation rate (Nottle et al., 1988; Venter and Greyling, 1994; Molle et al., 1995), but the effectiveness of relatively short-term (7 days or less) supplementation during the luteal phase for increasing ovulation rate has only been described in several studies in ewes (Stewart and Oldham, 1986; Nottle et al., 1990). In fact, constant nutritional supplementation for 7 days failed to increase ovulation rate in goats (Haruna et al., 2009). A certain period of increased metabolic status stimulating the emergence and growth of ovulatory follicles is likely to be required in a strategy for the promotion of the ovarian performance by short-term nutritional stimulus. Estrogens and growth hormones cause an increase in total plasma proteins owing to their anabolic effects (Kaneko, 1989).

Larson and Kendall (1957) reported an increased level of albumin and total protein in the blood of cows at the time of estrus. The increased levels of total protein in the ewe serum during estrus may be derived from increased metabolic activity while the ewe is under the influence of higher levels of estrogen. It is known that blood cholesterol concentrations and steroid hormones synthesis are positively related to energy intake and health of animals (Velhankar, 1973), while lower cholesterol and glucose concentrations after calving have been associated with an increased number of days from calving to conception (Kappel et al., 1984). Rabiee and Lean (2000) suggested that glucose may promote cholesterol uptake into the ovarian cells and vice versa. Similarly, high serum cholesterol concentrations in late pregnancy and early post partum were associated with the earliest resumption of ovarian cyclicity (Guedon et al., 1999).

McClure et al. (1978) and Funston et al. (1995) demonstrated that depletion of glucose availability in ewes through the use of 2 deoxyglucose suppressed pituitary release of LH and prevented expression of estrus and corpus luteum formation. Thus, glucose may be a regulator of GnRH release (Randel, 1990; Short et al., 1990). Concentrations of plasma cholesterol were positively associated with expression of estrus at first ovulation, interval from calving to conception, and likelihood of conception and pregnancy (Westwood et al., 2002). Hypoglycaemia is one indicator of negative energy balance (NEB) at the beginning of lactation and ketosis, reflecting discrepancy between metabolic demands and rate of gluconeogenesis in high yielding dairy cows (Fratric et al., 2007). The level of blood urea is a useful tool for estimating the protein nutritional status in ruminants, as it is readily affected by the dietary intake of protein and energy (Ide et al., 1966), in particular by the ratio between protein and energy (Carlsson and Bergström, 1994). In conclusion, the short intermittent energy supplement increases the number of ovulatory follicles and ovulation rate in subtropical ewes which might increase the reproductive performance of these animals.

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