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ARTICLE

Impact of short-term protein supplementation on estrus, ovarian activity, and blood metabolites in Ossimi ewes synchronized with PGF2 α analogue (*Cloprostenol*) in subtropics

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ABSTRACT

The aim was to elucidate the effects of short-term, high protein diet on ovarian activity and metabolic status in synchronized Ossimi ewes. Fourteen Ossimi ewes divided into a high protein (HPG; n = 7) and a control group (CG; n = 7). Estrous synchronized using two doses of Prostaglandin F2α (PGF2α) that were administered 10 days apart. For the five days before the second dose of PGF2-α, a high protein diet consisting of 20% crude protein was fed to the HPG and the CG was provided a maintenance diet. The estrus period was significantly longer and the ovulation rate was significantly higher in the HPG as compared to the CG ($P < 0.05$). A significantly longer ovulation time and larger diameter ovulatory follicles were observed in the HPG ($P < 0.05$). A high protein diet had a significant effect on the number of recruited follicles and the diameter of the ovulatory follicle ($P < 0.05$). Significantly higher levels of estradiaol-17β, total protein, albumin, total cholesterol, blood urea, and glucose detected in the HPG as compared to CG ewes ($P < 0.05$). It is concluded that short-term, high protein flushing may improve estrus expression, ovarian activity, and metabolic status in PGF2α analog synchronized Ossimi ewes.

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Blood metabolites; Follicular dynamic; Protein; Ossimi ewes; Sex hormone

1. Introduction

Nutrition plays a critical role in controlling the reproductive performance of farm animals and affects features of reproduction by two pathways (Scaramuzzi et al. 2006). One pathway acts through the endocrine system (GnRH, FSH-LH, and estradiol). Scaramuzzi et al. (2006) in which reproduction is affected by nutritional inputs that act directly on the ovary and the ovarian follicles via changes in metabolic modulatory systems (insulin-

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glucose, leptin and growth hormone, and growth factors) proposed a second pathway. Stimulation of these intra-follicular systems suppresses follicular oestradiol production. These direct actions on the follicle reduce negative feedback on the hypothalamic-pituitary system and lead to increased FSH secretion that results in stimulation of folliculogenesis. In sheep, static body condition leads to changes in FSH secretion, which affect follicular dynamics and ovulation rate patterns (Viñoles et al. 1999). Protein supplementation (Wiley et al. 1991; Noguchi 2000) may modify insulin, the IGF system, and leptin (Snyder et al. 1999; Sansinanea et al. 2001), which are mediators of energy balance and reproductive function that are influenced by nutrition. Integration of nutritional status and reproduction appears to be affected by the hypothalamo-hypophyseal- ovarian axis (Keisler and Lucy 1996; Wiltbank et al. 2002). In sheep, follicle populations are sensitive to nutritional input and folliculogenesis and the ovulation rate can be increased by nutritional alterations (Downing et al. 1995b). Nutritional manipulation of reproduction is an inexpensive management tool that can be used to control ovulation rate and litter size. This management tool is particularly effective for low-cost, extensive production systems used in marginal environments such as the semi-arid Mediterranean and hill farming regions of the world (Martin et al. 2004) and in the Ossimi breed, which is characterized by low fecundity (Aboul-Naga 1985).

We hypothesized that supplementing the diet of non-pregnant ewes with large amounts of protein for a short duration would alter peripheral steroid hormones, follicular dynamics, and blood metabolites. Therefore, this study evaluated the effects of short-term, high protein supplementation on the plasma concentration of steroid hormones and metabolites and on follicular dynamics in subtropical ewes.

2. Materials and methods

2.1. Experimental design and animal management

The experiment was conducted during the spring breeding season (March–May) (Ali et al. 2006), which followed protocols approved by the Ethics Committee on Animal Experimentation of Assiut University, Faculty of Veterinary Medicine. Ewes belonged to the Research and Production Animal Farm, Department of Animal Production, Faculty of Agriculture, Assiut University, located in Upper Egypt. Fourteen apparently healthy Ossimi ewes 4–5 years of age, pluripara, non-suckling, with an average body weight (mean ± SD) of 45.8 ± 0.2 kg were included in this study. The ambient temperature and relative humidity inside the animal farm ranged from 26.6 to 33.4 °C and 35 to 31 %, respectively. Ewes were kept indoors in individual pens and food and water were provided *ad libitum*. For adaptation, all ewes were fed the same maintenance diet for a 3-week period. Next, the animals were randomly assigned into two equal groups: the high protein group (HPG, n = 7, 20% crude protein (CP) and the control group (CG, n = 7, 9.5% CP). The high protein group received a short-term, high protein diet (over the five days prior to the second dose of PGF2α), while the CG received a maintenance diet throughout the experimental period. Feed was mixed daily and all ewes received two meals of equal allotments at 08:00 and 17:00 h according to National Research Council guidelines (NRC 1985) and the refusals were removed and weighed each time. Table (1) shows the chemical composition and ingredients of the experimental diets.

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

Item	Control group (CG)	High protein Group (HPG)
Ingredient, %		
Yellow corn	32.00	5.00
Cotton seed meal, undecorticated	6.00	45.20
Wheat bran	28.20	18.00
Soybean meal	2.00	10.00
Premix*	0.30	0.30
Ground lime stone	1.00	1.00
Salt	0.50	0.50
Wheat straw	30.00	20.00
Total	100	100
Analyzed chemical composition (% unless stated)		
ME Mcal/kg **	2.04	2.04
ME MJ/kg	8.54	8.54
Crude protein	9.5	20.00
Crude fiber	16.48	19.79
Ether Extract	2.52	3.35
Nitrogen free extract	66.41	51.40
Ash	5.02	5.42
Calcium	0.42	0.51
Phosphorus	0.54	0.74

*Ingredient and nutrient composition are reported on as-fed basis.
Control group(CG, 9.5% CP), high protein group (HPG; 20 % CP)
*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 $caco_3$.
** ME of diet ingredients was calculated based on NRC (1985) feed composition tables.

The protein level for the CG was reduced by increasing wheat straw at the expense of cottonseed meal, soybean, and wheat bran. Feed samples from experimental diets were collected, thoroughly mixed, and ground, followed by chemical analysis of each feed separately. Daily feed intake, initial and final body weight were recorded during the experimental period.

The composition of the HPG diet contained 20% crude protein, which was nearly double the amount of crude protein in the CG diet. The mean crude protein intake was 117.65 g/day and 253.65 g/day for the CG and the HPG, respectively.

2.2. Estrus synchronization and observation of animal behavior

Two intramuscular injection of the PGF2α analog cloprostenol at a concentration of 250 µg/mL (1 mL Juramate®, Jurox Pty. Ltd., Australia) were administered to synchronize estrus in all ewes, with the two treatments spaced apart by 10 days. Estrus behavior was detected after the second dose of PGF2α with 6 h interval for 5 days or until signs of heat subsided using active teaser rams (n = 3). Duration of estrus was defined as the interval between the onset (the time when the ewe first stood to be mounted by the ram) and the end (recognized by either disallowance of mounting by the teaser ram or unfollowing of teaser ram to ewe) of estrus.

2.3. Monitoring of follicular and corpus luteum development

Animals with genital tract diseases were not included in this study. The same operator performed daily trans-rectal ultrasound scanning that started on the first day of

supplementation of the high protein diet until the tenth day after ovulation. Near the expected time of ovulation, ewes were examined twice per day for the precise determination of ovulation. All examinations were conducted in the standing position using an ultrasound scanner equipped with a 6–8 MHz linear transducer (Pie-medical, 100 LC, Holland). For easy manipulation of the transducer in the rectum, it was fitted with the connector. The number, diameter, and relative position of all follicles ≥2 mm in diameter and corpora lutea were detected during each examination. Additionally, these characterized follicles were further evaluated by retrospective evaluation of ovarian sketches that provided topographical and dimensional changes of each follicle and corpus luteum (CL). When a follicle or Cl was not spherical, a mean diameter of two dimensions was recorded. Ovulation was recorded and was considered to have occurred when a large growing antral follicle that had been identified and followed for several days was no longer observed (Ginther et al. 1995).

2.4. Blood sampling and biochemical and hormonal analysis

On a daily basis each morning, blood samples were collected by jugular venipuncture during the treatment period and for 12 days after the second PGF2α dose. Blood samples were centrifuged at 2,000 × g for 20 min, then serum was harvested and stored at −20°C until assays were performed. Blood metabolites (total protein, albumin, globulin, total cholesterol, glucose, and urea) were analyzed with a spectrophotometer (Unico, USA) using commercial test kits (Spinreact, Spain). The methods used for the biochemical analysis were performed as previously described (Young 2001). The reaction temperature used for metabolite analysis was 25–37°C, which was based on the instructions provided on the enclosed pamphlet of Spinreact Company. Estradiol-17β (E2) and progesterone (P4) concentrations were determined using the direct ELISA technique. Kits were provided by Diagnostic System Laboratory Co. (DSL, Catalogue No. 3900, USA). The intra- and inter-assay coefficient of variation for estrogen was 4.8% and 9.2%, respectively, and 3.6% and 12.43%, respectively, for progesterone. The sensitivity of the assay was 2 pg for E2 and 0.12 ng for P4.

2.5. Statistical analysis

Data were statistically analyzed using SAS (1996) software. All variables were tested for normal distribution tested using the Kolmogorov–Smirnov normality test. All parameters were normally distributed. Comparison of mean values between the treatment and control group was performed using the independent-samples T-test on each time point. Analysis of variance with repeated measurements of the general linear model using the Bonferroni multiple comparison tests were used to investigate the influence of time on each variable in each group. Probability values less than 0.05 ($P < 0.05$) were considered significant. Results are presented as means ± SE.

3. Results

3.1. Body weight

For the ewes, the initial and final body weight, daily weight gain, and feed conversion were similar (P > 0.05) during the feeding period and did not differ (P > 0.05) at any time during treatment (Table 2). The high protein diet significantly increased the daily feed intake (1.268.29 ± 1.22 g/head/day, P < 0.001) as compared to the CG (1.238.43 ± 0.97 g/head/day).

3.2. Estrus features

The influence of short-term protein supplementation on the onset and duration of estrus and time of ovulation in estrus-synchronized Ossimi ewes that received two doses of PGF2α analog is presented in Table (2). Estrus initiated numerically not statistically (P > 0.05) later on the treated group HPG but lasted significantly longer in the HPG than in the CG (20.8 ± 3.2 h vs. 14.1 ± 3.4 h; P < 0.05). Ovulation occurred later with larger diameter ovulatory follicles in the HPG than in CG ewes (P < 0.05).

3.3. Follicular growth and dynamics

Dietary short-term CP significantly increased the number of recruited follicles (2–2.9 mm in diameter), medium-sized follicles (3–5 mm in diameter) (P < 0.05) and had a significant effect on the growth rate/day (HPG: 1.2 ± 0.01 mm; CG: 0.8 ± 0.02 mm, P < 0.05) and the diameter of the ovulatory follicle (HPG: 6.3 ± 0.2 mm; CG: 5.1 ± 0.2 mm, P < 0.05, Table 3 and Figures 1 and 2). Moreover, four of seven (57.1%) and six of seven (85.7%) ewes had ovulation for the CG and the HPG, respectively. There was a significantly higher ovulation rate in the HPG than in the CG (HPG: 2.5 ± 0.3; CG: 1.1 ± 0.2 mm, P < 0.05). There were no significant differences in the diameter of corpus luteum or its regression rate between ewes in the HPG and the CG.

Table 2. Growth performance of ewes fed the experimental diets.

Item	CG	HPG	P
Initial body weight, kg	45.85 ± 0.07	45.97 ± 0.07	0.3
Final Body weight, kg	46.32 ± 0.06	46.49 ± 0.08	0.13
Daily feed intake, g	1238.43 ± 0.97	1268.29 ± 1.22	<0.001
Average daily weight gain, g	94.29 ± 4.28	104.29 ± 5.28	0.17
Feed conversion	13.31 ± 0.62	12.38 ± 0.68	0.33

Table 3. The effect of short protein supplementation on the onset and duration of estrus and time of ovulation in control and high protein groups of ewes treated with double doses of PGF2α to synchronize estrus (n = 7 for each, mean ± SEM).

Groups	Onset of estrus (h)	Duration of estrus (h)	End of estrus (h)	Time of ovulation (h)	Ovulatory follicle Ø (mm)	Number of emerged follicles
CG	41.3 ± 4.9	14.1 ± 3.4^{b}	55.4 ± 4.2^{b}	40.5 ± 5.6^{b}	5.1 ± 0.2^{b}	3.1 ± 0.3^{b}
HPG	46.2 ± 3.7	20.8 ± 3.2^{a}	66.0 ± 3.5^{a}	61.3 ± 3.8^{a}	6.3 ± 0.2^{a}	5.4 ± 0.3^{a}
P	0.08	0.02	0.01	0.0001	0.03	0.01

The values with different superscripts (a,b) in the same column differ significantly (P < 0.05)
Onset of estrus in relation to the second dose of PGF2α injection

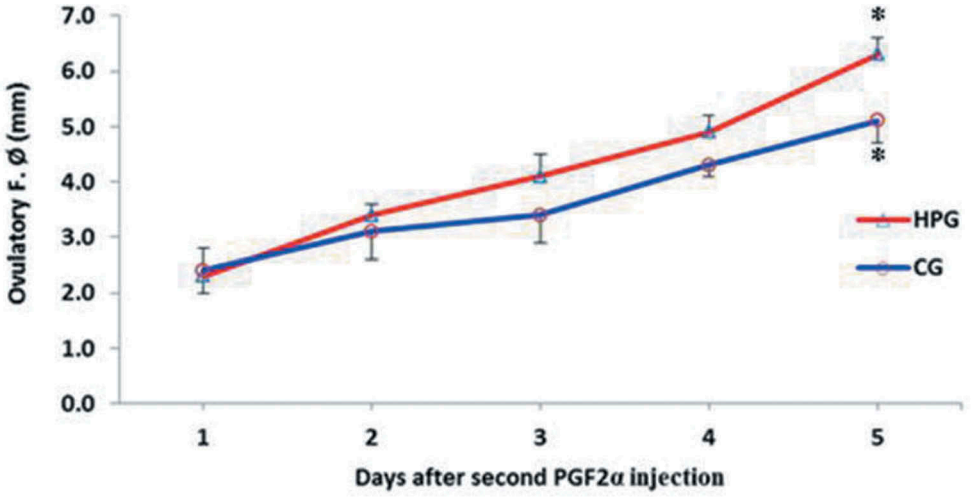

Figure 1. Ovulatory follicle diameter (mm) following second dose of PGF2α in Ossimi ewes (HPG and CG) *, ovulatory follicle higher in HPG at days 4 and 5 after the second dose of PGF2α when compared to NPG. * Significant level was set at $P < 0.05$.

3.4. Steroid hormone profiles

Collectively, there was no difference in serum P4 levels between the CG and the HPG during the experiment ($P > 0.05$). On the other hand, it was observed that, serum estradiaol-17β levels were higher in the HPG ($P < 0.05$) than in the CG following the second dose of the PGF2α analog (Figure 3). There were no correlations between CL diameter and progesterone concentration ($P > 0.05$, $r = 0.04$).

3.5. Blood metabolites

There were significant increases ($P < 0.05$) in the levels of total protein, albumin, total cholesterol, blood urea, and glucose during the first three days following the second dose of PGF2α analog in the HPG as compared to the CG group. No significant difference in globulin concentration was observed between both groups ($P > 0.05$, Table 4).

4. Discussion

In the present study, no marked changes were observed in ewe body weight and growth performance indices between groups. An acute or immediate effect of nutrition is an increase in the ovulation rate with no changes in live weight or body condition (Oldham and Lindsay 1984; Somchit et al. 2007; Da Costa et al. 2011). However, it was reported that supplying post-parturient ewes with a high level of undegradable protein in the early post-parturient period allowed them to consume more feed and gain more body weight (Kridli et al. 2001; Haddad et al. 2005). The results of the present study disagree with previous findings (Kridli et al. 2001; Haddad et al. 2005); the level and duration of protein supplementation, suckling as well as postpartum period may be

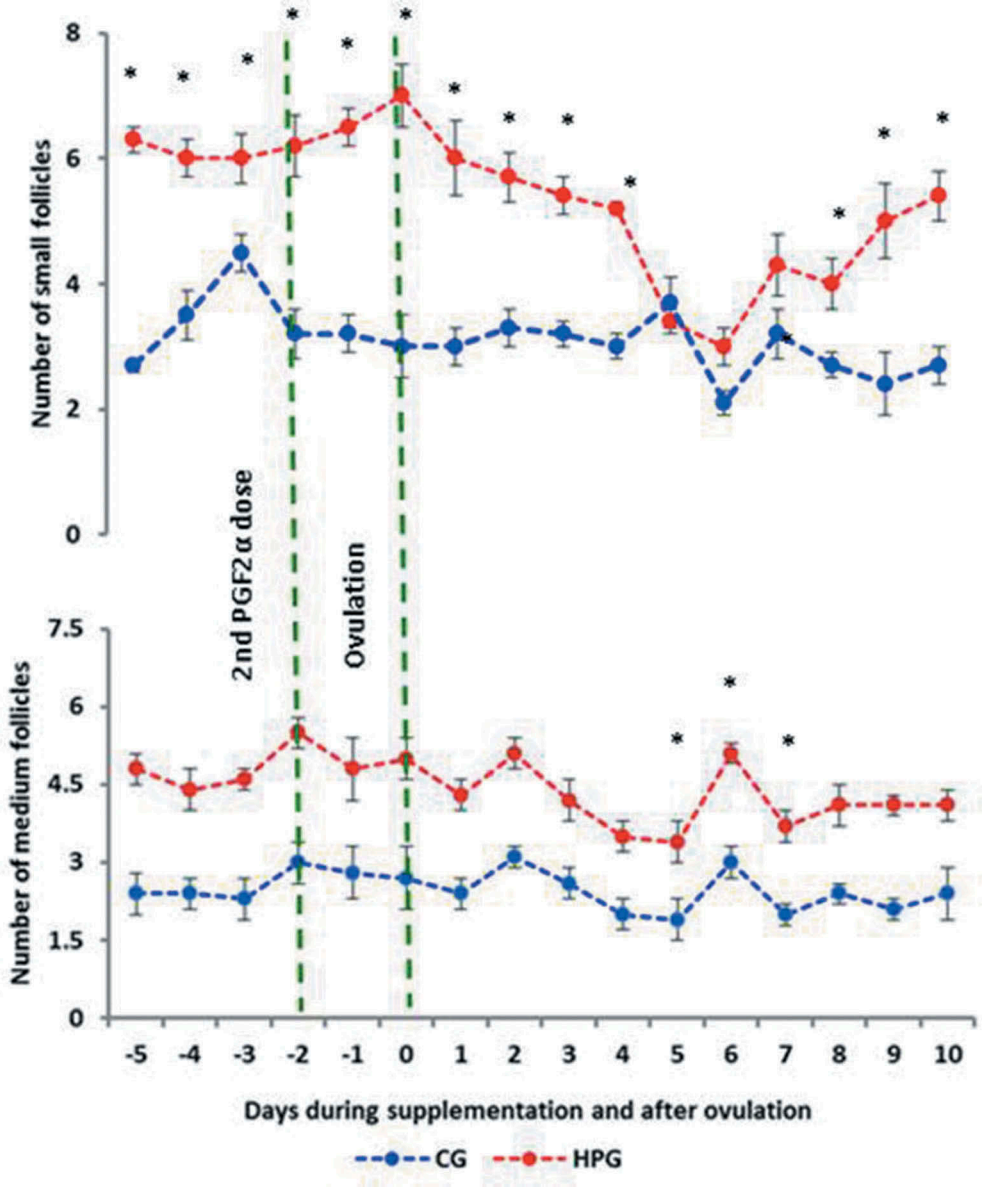

Figure 2. Follicular number during supplementation and after second PGF2α dose in high protein ewes group (HG, n = 7) and in maintenance one (CG, n = 7), values in mean ± SEM, * Significant level was set at $P < 0.05$.

crucial in this concern and could be the main cause of this discrepancy between our results and previous reports.

In the current study, the significant increase in the number of recruited follicles, growth rate/day, diameter of the ovulatory follicle, and ovulation rate in the HPG coincides with previous studies (Waghorn et al. 1990; Somchit et al. 2007; Meza-Herrera et al. 2008). The ovulation rate was increased by providing excess dietary CP for 5–8 days before anticipated

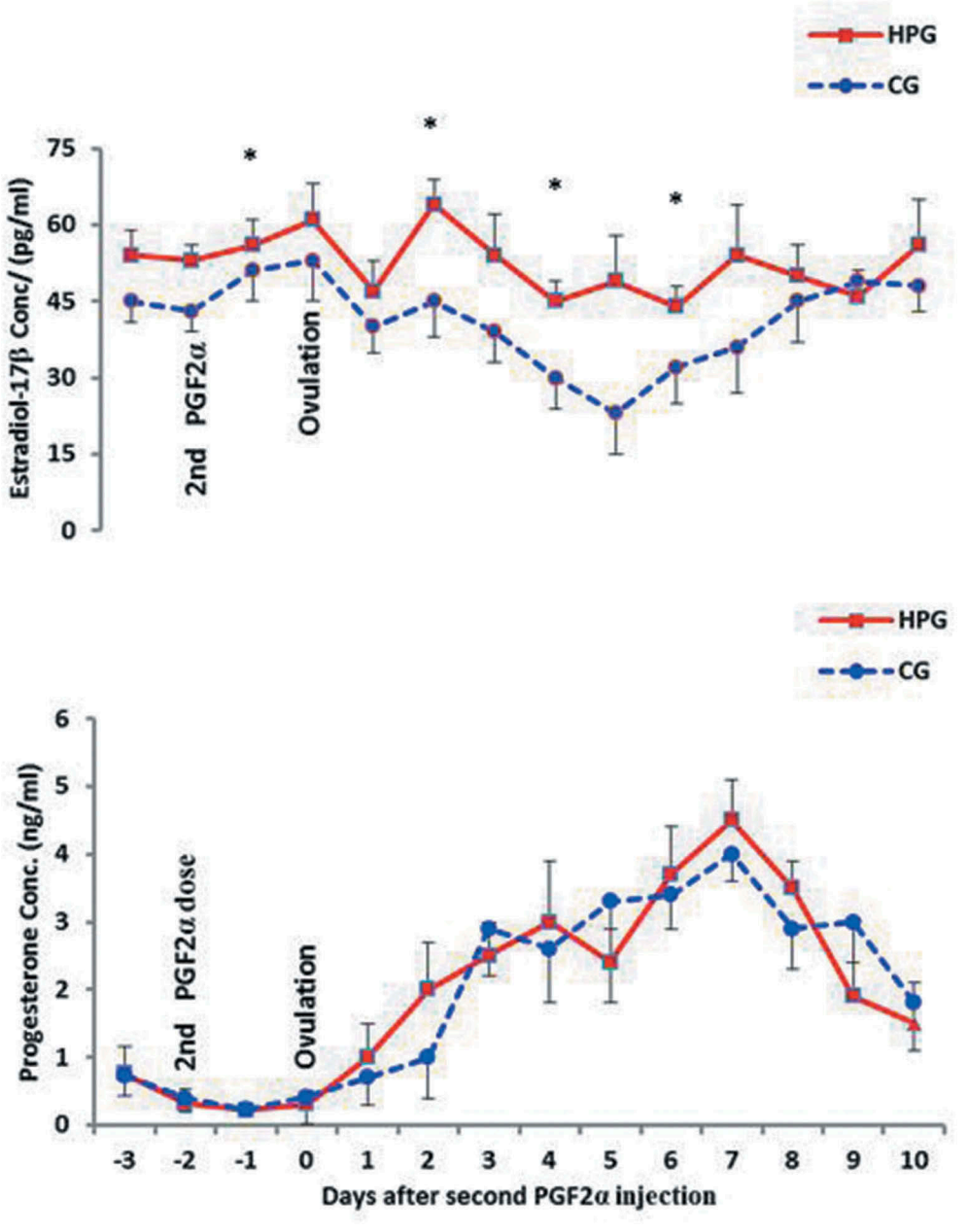

Figure 3. Serum level of estrogen (pg/ml) and progesterone (ng/ml) after second PGF2α injection and around ovulation (Day 0) in ossimi ewes fed on short high protein diets (HPG) compared with control ones (CG). * Significant level was set at $P < 0.05$.

estrus (i.e. beginning of the mid-luteal phase) (Oldham and Lindsay 1984; Smith 1988; Smith and Stewart 1990). Increased levels of protein in the diet have been associated with increased ovulation rates and increased levels of circulating FSH during the latter half of the estrus cycle (Knight et al. 1975; Davis et al. 1981; Viñoles 2003). Nutritional flushing was

Table 4. Changes in blood metabolites after second dose of PGF2α analog in both control and high protein groups of Ossimi ewes ((n = 7 for each, mean ± SEM).

	Group	Total protein g/L	Albumin g/L	Globulin g/L	Glucose mg/dl	T-Cholesterol mg/dl	Urea mg/dl
Day 1	CG	7.8 ± 0.9[b]	2.7 ± 0.2[b]	5.1 ± 1.0	53.5 ± 6.5[b]	57.1 ± 5.1[b]	63 ± 4.1
	HP	10.9 ± 1.2[a]	4.6 ± 0.2[a]	6.3 ± 0.9	79.5 ± 4.9[a]	86.4 ± 4.6[a]	62 ± 3.9
	P	0.003	0.02	0.07	0.004	0.002	0.37
Day 2	NPG	5.8 ± 0.9[b]	1.9 ± 0.3[b]	5.9 ± 1.1	43.1 ± 4.5[b]	47.9 ± 2.9[b]	53 ± 2.0
	HPG	9.4 ± 1.7[a]	3.4 ± 0.3[a]	6.0 ± 0.8	69.3 ± 2.1[a]	76.1 ± 3.2[a]	56 ± 1.9
	P	0.001	0.01	0.22	0.001	0.001	0.12
Day 3	CG	5.2 ± 0.7[b]	1.4 ± 0.2[b]	4.8 ± 1.8	47.7 ± 2.5[b]	55.1 ± 9.6[b]	65 ± 2.7 [b]
	HPG	9.6 ± 1.9[a]	4.6 ± 0.2[a]	5.0 ± 2.2	99.5 ± 10.3[a]	86.4 ± 9.6[a]	76 ± 2.5 [a]
	P	0.003	0.02	0.17	0.005	0.01	0.02
Day 4	CG	6.8 ± 0.7[b]	2.8 ± 0.3[b]	4.6 ± 1.0	43.5 ± 6.4[b]	49.1 ± 7.8[b]	53 ± 4.4 [b]
	HPG	10.1 ± 0.9[a]	5.1 ± 0.4[a]	5.0 ± 0.9	89.5 ± 5.7[a]	86.4 ± 9.2[a]	84 ± 4.3 [a]
	P	0.001	0.01	0.08	0.001	0.0001	0.002
Day 5	CG	7.8 ± 0.9[b]	2.7 ± 0.2[b]	5.8 ± 1.7	53.5 ± 6.5	57.1 ± 9.6[b]	61 ± 3.1 [b]
	HPG	10.9 ± 2.2[a]	4.6 ± 0.2[a]	6.3 ± 1.6	59.5 ± 5.9	86.4 ± 9.6[a]	89 ± 3.2 [a]
	P	0.002	0.01	0.11	0.34	0.03	0.003

Means with different superscripts (a, b) in the same column (for each day separately) differ significantly (P < 0.05)
HPG: high protein group (20% CP)
CG: Control group (9.5% CP).

found to alter the blood concentration of certain reproductive hormones using the short-term flushing model; a transient increase in FSH and a decrease in estradiol concentration in the blood (Scaramuzzi et al. 2006). In the ovary, the effect of nutrition is stimulation of folliculogenesis (Munoz-Gutierrez et al. 2002). The consequences of these direct actions on the follicle is reduced negative feedback on the hypothalamic-pituitary system and increased FSH secretion that leads to a stimulation of follicle maturation and transition to a larger follicular category. However, several studies demonstrated that the concentration of glucose and metabolic hormones reached peak values two or three days after the start of a high level of feeding then decreased while nutritional supplementation continued for six or seven days in ewes (Viñoles et al. 2005) and in goats (Haruna et al. 2009). Our findings clearly indicate that increases in the number of follicles may not only be due to changes in blood levels of progesterone and estradiol-17β but also changes in glucose concentrations. Several lines of evidence suggest that increases in blood glucose and insulin levels regulate glucose availability at the follicular level and during folliculogenesis in ewes (Munoz-Gutierrez et al. 2002; Letelier et al. 2008). Since flushing with soybean meal results in excess protein allowance (NRC 1985), part of the extra amino acids may be converted to glucose by gluconeogenesis. Also, in a previous study done by Molle et al. (1995), soybean flushing improves ovulation rate in the high protein group compared to CG (1.60 VS 1.18 CL per ewe, respectively). Moreover, it was reported that protein-rich supplements have a positive effect on the ovulation rate in ewes (Smith 1988; Rhind 1993). The increase in the number and size of follicles and the increase in ovulation rate (OR) positively correlated with a change in concentration of amino acids, particularly the branched-chain amino acids (BCAA) (Waghorn et al. 1990). Infusions of BCAA increased OR in ewes (Downing et al. 1995a). In the current study, protein level had no effect on either corpus luteum diameter or its regression rate and there was no correlation between the Cl diameter and progesterone concentration similar to previous observations (Landau et al. 1996; Viñoles et al. 2000; Somchit et al. 2013). However, Jing et al. (2017) observed a high level of progesterone in the energy and protein

supplemented group. This disagreement between our results and the results of Jing et al. (2017) may have been due to the age of the ewes and type and duration of supplements.

In the current study, the significantly high estradiol-17β concentration could have been due to the higher number of ovulatory follicles in the HPG as compared to the CG, similar to previous reports (Waghorn et al. 1990; Scaramuzzi et al. 2006).

Increased levels of serum total protein and cholesterol in the ewe may result from the anabolic effects of estrogens (Kaneko 1989; Hussein 1996). Our study showed that the total cholesterol concentration was high in the HPG, which is consistent with many previous studies (Kridli et al. 2001; Haddad et al. 2005; Scaramuzzi et al. 2006). A key factor of normal ovarian function is glucose availability and most mammals obtain glucose from their diet; however, ruminants obtain low amounts of glucose from dietary sources. Biosynthesis of glucose from dietary precursors is the main source of glucose for ruminants. The main precursors of gluconeogenesis are propionic acid (a product of anaerobic fermentation of carbohydrates in the rumen) and gluconeogenic amino acids absorbed from the small intestine (Overton et al. 1999).

The level of urea in the blood 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 the current study, there was a significant increase in the blood urea concentration, similar to a previous study (Mahmoud et al. 2014). A high level of urea may have a negative effect on second ovulation rate in treated ewes (not tested in this study) due to the negative effect of ammonia on the reproductive tract (Fletcher 1981; Garnsworthy et al. 2008).

According to Scaramuzzi et al. (2006), flushing with high energy improves energy balance, leads to increased leptin and insulin concentrations in the blood, and increases the uptake of glucose; these changes appear to directly affect the ovary and are associated with increased ovulation rates in sheep. Negative effects of high protein diets on fertility were previously reported for ewes (Wallace et al. 1994; McEvoy et al. 1997; Tur et al. 2017), beef heifers (Gath et al. 2012), and dairy cows (Butler 1998, 2000). Excess dietary protein fed to ewes reduced fertility and increased embryonic loss, both in vivo and in vitro (McEvoy et al. 1997). Several studies (Howard et al. 1987; Carroll et al. 1988) did not find that diets with high crude protein (20%) had a negative effect on the reproductive performance of early lactation cows. The negative effect of a high protein diet varies according to protein content, protein degradability, non-protein nitrogen, and energy of the diet. Sperm motion through the oviduct, oocyte maturation, fertilization, and early embryonic survival may be affected by toxic components of nitrogen metabolism, particularly ammonium ions. We assumed that the negative effect of a high protein diet could occur in the postovulatory phase during fertilization and embryonic growth when associated with a negative energy balance. In addition, a high protein and high energy diet may increase nitrogen retention and could have negative effects on fertility; thus, a medium level of protein is better than high or low protein levels. In our study, the negative effect of a high protein diet did not occur because of the short time of high protein feeding and all ewes were in positive energy balance. Additionally, we did not investigate the effect of protein supplementation during fertilization and embryonic growth and on subsequent fertility parameters.

5. Conclusion

Short-term (5 days) high protein supplementation had a significant impact on ovarian activity and certain biochemical parameters in PGF2α synchronized Ossimi ewes that were in a state of positive energy balance and were not post-parturient. These effects included an increased follicular population, increased diameter of ovulatory follicles, and increased ovulation rate. High protein supplementation increased the level of cholesterol, glucose, total protein, blood urea, and estradiol 17-β hormone. However, there were no significant changes in live body weight and progesterone. Thus, the gonadal response to short-term nutritional supplementation appears to be associated with metabolic or nutritional signals to the follicle and specifically insulin rather than with changes in body weight. In conclusion, short-term flushing of ewes with a high-protein diet (five days before the second dose of PGF2α) concomitant with PGF2α synchronization increased estrous activity and improved reproductive performance of Ossimi ewes. Further studies of inseminated ewes are needed to examine the acute and short-term effect of protein supplementation on subsequent fertility parameters.

Statement of animal rights

All Institutional and National Guidelines for the care and use of animals were followed according to the Egyptian Medical Research Ethics Committee (no. 14 – 126).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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