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Protein based flushing related blood urea nitrogen effects on ovarian response, embryo recovery and embryo quality in superovulated ewes

İrfan Tur [a, *], Dursun Ali Dínç [b], Ahmet Semacan [b]

[a] Department of Molecular Biology and Genetics, Izmir Institute of Technology, Gülbahçe Campus, Urla, Izmir, 35430, Turkey
[b] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Selçuk University, Alaaddin Keykubat Campus, Selçuklu, Konya, 42003, Turkey

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ABSTRACT

The present study is the first report that evaluates effects of nutritional effects of flushing with differing diet crude protein ratios on blood urea nitrogen (BUN) levels, related some reproductive parameters and embryo quality in ewe. During mating season, before synchronization protocol ewes were fed on alfalfa hay and additive concentrate feeding as flushing. Intra vaginal FGA containing sponges applied for 12 days for the purpose of synchronization and pFSH was administered by 8 declining doses for the purpose of superovulation.

Uterus was flushed in the morning of the seventh day of mating and embryos were collected surgically. Collected embryos were qualified according to IETS criterion. There is no dependency found between BUN values measured at different days and at different diet crude protein concentrations. An increase in uterine pH levels due to increasing protein amounts was observed but this increase was not significant among groups. Ovarian function was evaluated by ovarian responses (CL + large follicle) showed difference between groups ($p < 0.05$) and the lowest protein intake group gave highest ovarian response. In addition, embryo recovery rates revealed difference between groups ($p < 0.05$) and it was observed that the lowest ovarian response group showed the highest rates of embryo recovery. It is concluded that, in some Anatolian native sheep breeds, the application of diet flushing with different crude protein concentrates influence ovarian responses and embryo recovery rates but has no effect on BUN levels; uterus physiology or embryonic quality.

1. Introduction

The present study aims to investigate the effects of nutritional protein flushing on BUN levels, some uterus physiological parameters, ovarian responses and embryonic quality in some native Anatolian ewe breeds during the breeding season. Nutrition affects all reproductive processes from gametogenesis to puberty and further in both female and male specimens [1]. A wide array of researches have been focused on revealing both environmental factors and mechanisms at play. While studies related to ewe are not sufficient, there are numerous studies conducted on dairy cows, focusing mostly on which relationship between diet protein levels and milk production; BUN levels and decreased fertility [2–7]. The general point of view attends the association between reproduction and nutrition from the frame of energy balance and research models were based on energy maintenance [1,8]. Also the majority of the studies [9,10] evaluating different protein concentrations indicate that increasing diet protein levels would result in decreased conception rates.

In natural conditions, survival of the species critically associated with seasonal feeding and reproduction. Along with the domestication, basically, reproduction capacity of animals always put forward with the aim to increase capacity. Thus new approaches to improve reproductive capacity have been the subject of new studies. Although it is mentioned especially with the ewe, flushing feeding before mating and seasonal reproduction is an important part of the reproductive management in seasonally reproducing animals. Researcher studied feeding flushing in ewe and cow mostly in terms of energy maintenance. They concluded in consensus that high and low energy diets are detrimental to oocyte and embryo development and related production processes [10–15]. But the effect and efficiency level of diet protein levels on reproductive physiology is remained elusive.

* Corresponding author.
E-mail addresses: irfantur@yahoo.com (İ. Tur), dadinc@selcuk.edu.tr (D.A. Dínç), asemacan@selcuk.edu.tr (A. Semacan).

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Proteins and providing amino acids are vital for growth, metabolism, and reproduction. Ruminants have the ability to synthesize amino acids in rumen microflora and produce essential amino acids from non-protein nitrogen resources. Ruminants also have the ability to decrease protein loss by recycling urea [11]. But they also need structural proteins for urea cycle and microflora to work well.

The relative importance of flushing on reproductive efficiency illustrates the difficulty of determining protein and energy requirements for maximum reproductive performance. However, Torell DT et al. (1972) [9] concluded that notwithstanding protein and energy, on the basis of maximum live weight gain resulted in maximum reproductive performance. High urea in circulation and milk associated with excess protein intake and probably energy shortage [13]. On the other hand, increase in energy and protein in diet, raise nitrogen retention in ruminants and results in urea increase, which is one of the main actors of this research. Urea is a relatively small molecule (about 60 g/mole) that can freely move between cell membranes, therefore, circulation of urea between blood and other tissues is especially high [6]. Also urea is an important part of the protein metabolism and nitrogen cycle in ruminants. Again, it is clearly stated that increasing BUN levels are related to decreased fertility and embryonic loss [16]. Thus it can be said that direct effect of high protein intake on uterus may be detrimental for reproductive processes because of high urea. Toxic components of nitrogen metabolism, most particularly ammonium ions may affect sperm motion through oviduct; oocyte maturation; fertilization and early embryonic survival. It is reported that these effects may be modulated by uterine pH [10). By the way, McEvoy et al. [17] concluded that dietary changes which elevate ovulatory responses can be detrimental for embryo quality. In addition, it is accepted that high energy level effects oocyte and embryo quality may have negatively [18]. As a result, though there are many unclear definitions, it is clear that improving energy and protein intake by supplemental feeding is detrimental for oocyte and embryo. In comparison with more clear evidence of nutritional supplementation to enhance energy maintenance, protein based effect of reproductive efficiency is not clearly associated with BUN levels. Thus, the current study has two main objectives: 1) to test effects of different crude protein ratio supplemental feeding on reproductive efficiency directly and 2) to determine indirect effect of BUN on uterine physiology and reproductive efficiency.

2. Material and methods

The study was carried out during major mating season (late in summer and early in autumn) when most of the ewe manifest sexual activity in Turkey. The animals were maintained at the Animal Husbandry Unit of Selçuk University in Konya, Turkey which is located in Central Anatolia Region and at latitude 38° 1′ N, longitude 32° 30′ E and 1177 m of above sea level. Application procedure was sanctioned by Commission for Ethics on Animal Experiments of Selçuk University. Ewe breeds that are native to Anatolia (Daglıç, Herik, and Norduz) are kept under standard husbandry practices.

2.1. Experimental design

In the present study, 63 non-pregnant and cycling ewes of Anatolian strains aged 3–4 years and same body condition score (BCS:3) were used. Particularly the ewes that have undergone a successful pregnancy prior to the study were scanned for and selected. The ewes were randomly allocated in equal number ($n = 21$) to three different dietary groups. Also, the ewes were housed in slatted indoor housing and there was no direct and close male effect surrounding. The ewes were fed by dry alfalfa hay and sunflower meal before feeding flushing. With the onset of the study, in addition to ad libitum alfalfa hay, 700 g of concentrate mixture were provided per ewes per day as daily diet (nutritional composition of concentrate mixture shown in Table 1). Each group was allocated into one of the following diet groups depending concentrate mixture: (i) high protein diet (HP), (ii) medium protein (control) diet (MP) and (iii) low protein diet (LP). For optimal condition adequate open space was provided for all ewes. Feeding flushing procedure was maintained until embryo recovery.

2.2. Estrus synchronization and superovulation

The synchronization protocol was initiated 15 days after feeding flushing (see Table 2). To synchronize estrus, intra vaginal sponge containing 20 mg FGA were administered (Chronogest CR®, Intervet Productions SA., France) for 12 days [19,20]. Examination coupled with injection of a prostaglandin analog 0,392 mg (2 ml) tiaprost trometamol (Iliren®, Intervet) IM 72 h prior to sponge withdrawal. Superovulatory treatment was the same for all ewes and was initiated 4 days prior to mating. Each ewe was implemented with 200 mg pFSH (Folltropin-V®, Bioniche Animal Health, Canada) twice a day (morning and evening) in decreasing doses over a period of 4 days. The ewes were observed for estrous behavior by using a teaser rams for 30 min for every 6 h starting from 24 to 76 h after sponge withdrawal. Estrus positive ewes were filtered and immediately subjected to fertility-proven rams twice a day (morning and evening) for each. The vaginal mucosa of the ewe was controlled for ejaculation in order to assure that the mating is carried out. Ovarian responses and uterus physiology were evaluated on the 7th day of mating by laparotomy and the recovered cells were classified according to their quality.

2.3. Blood sampling and BUN analysis

Blood samples were collected respectively; at the beginning of feeding flushing procedure, at the beginning of synchronization; sponge withdrawal day; superovulation initiation day; on days of mating and surgical procedure day. In order to overcome stress on ewe, food and fresh water provisions were halted until the end of applications. Blood samples were collected from jugular vein of ewes into 10 ml plain vacuum tubes (Vacutainer®, Becton Dickinson, England). All samples were labeled and centrifuged at 3000 × g for 5 min after an hour following sampling. Extracted serum was deposited into 1.5 ml of Eppendorf tubes, labeled and stored at −20 °C until the analysis of urea nitrogen. Serum urea nitrogen concentration was measured using BT-3000 plus

Table 1
Nutritional compositions of feed.

Test	Group 1	Group 2	Group 3
Dry matter	%88,4	%88,6	%89,3
Ash	%7,4	%7,3	%7,1
Crude protein	**%12,17**	**%15,02**	**%17,92**
Acid detergent fiber (of dry matter)	%8,3	%11,6	%13,27
Neutral detergent fiber (of dry matter)	%19,52	%25,03	%26,8
[a]TDN (of dry matter)	%67,90	%65,07	%67,04
Fat (ether extract, of dry matter)	%2,46	%2,6	%3,98
Metabolic energy (Mcal/kg)	%2,47	%2,44	%2,49
Calcium	%1,44	%1,33	%1,15
Chloride	%0,3	%0,3	%0,3
Magnesium	%0,3	%0,4	%0,4
Phosphor	%0,2	%0,2	%0,3
Potassium	%0,7	%0,9	%0,8
Sodium	%0,2	%0,2	%0,2
Sulphur	%0,1	%0,2	%0,2

Bold indicates especially significant for the purpose of research's main topic.
[a] TDN: Total Digestible.

Table 2
Synchronization and super-stimulation protocols.

Synchronization and superovulation protocol	Morning	Evening
Day 0	Sponge administration	
Day 9	Tiaprost (150 μg) + 30 mg FSH	30 mg FSH
Day 10	30 mg FSH	25 mg FSH
Day 11	25 mg FSH	20 mg FSH
Day 12	20 mg FSH + sponge withdrawal	20 mg FSH
Day 13 and 14	Mating	Mating

Automated Photometric Clinical Biochemical Analyzer (Biotechnica Instruments®, Italy) in accordance with instructions provided by both manufacturer and attending specialist. BT 3000 analyzer utilizes spectrophotometric measurement using commercial colorimetric assay kit.

2.4. Determination of uterus pH and temperature

Uterus pH and temperature measurement were carried out shortly prior to flushing procedure from surgical fissure opened for washing uterus. The automatic pH and temperature reader (Testo 205®, AG Germany) was used and measurements were recorded manually for each animal.

2.5. Determination of ovarian response

The ovarian response was assessed by the number of luteal structure and large follicles (approximately larger than 3 mm) that were morphologically taken into account for each group. For each ewe, the number of corpora lutea (CL), large follicles and number of recovered ova were recorded. Accordingly, average rates of ovulated ewe (AO) were calculated by dividing the number of total luteal structures into number of ewes ($n = 21$). In general, a total of four or more follicles and luteal structures on both ovaries were accepted as super-stimulated for each ewe. Similarly, ewe with three or more CL on two ovaries were considered as super-ovulated and rates were calculated relatively [19]. Moreover, oocytes those have single cell and have one polar body were assumed as unfertilized oocyte (UFO). In addition, embryo recovery rate also was calculated by the evaluation of number of total cells collected and by CL numbers expected for recovery.

2.6. Embryo collection and cell number determination

On day 7 following mating, ewes were prepared for operation procedure. For premedication 0,22 mg/kg xylazine (Rompun® 2%, Bayer Healthcare AG, Germany) was used and ewes were anesthetized 15 min after premedication with injection of 2 mg/kg ketamine HCl (Ketasol® 10%, Richter Pharma, Austria) for surgical procedures. After a mid-ventral laparotomy, each uterine horn was flushed twice with 40 ml pre-warmed (37 °C) washing medium using Foley catheter in order to collect embryos. Expunged medium was sedimented by waiting for 30–45 min. Sediment mass was filtered and scanned thoroughly under light microscope (SXZ16, Olympus®) for embryos and evaluated for quality according to guidelines stated by Kanagawa et al. [21].

2.7. Embryo quality assessment

Recovered cell mass implies embryo, empty zona pellucida, unfertilized and one cell oocyte. Embryos that contain more than two cells were classified morphologically in accordance to the International Embryo Transfer Society (IETS) as grade 1, grade 2, grade 3 and grade 4. Both grade 1 and grade 2 embryos are classified as the good quality embryos that consist transferrable, freezable, compact morula and blastocyst and grade 3 and grade 4 embryos were classified as the low quality that consists untransferable and un-freezable or degenerated embryos in the various stage. Recovered cell mass was collected and quality observed under the stereomicroscope (SXZ18, Olympus®). Selected embryos were evaluated again thoroughly under the inverted microscope for quality assessment (IX3, Olympus®).

2.8. Statistical analyses

All groups were analyzed independently. BUN measurements, temperature and pH changes were analyzed by repeating-measures of ANOVA and Kruskal Wallis Variance Analyses. Moreover, results of Mauchy's spherical tests showed that sphering attained for BUN measurements for the time course. Chi-square analysis was used for comparison of values for ovarian response, super-stimulation, superovulation, embryo recovery rates and embryonic quality. Results were presented as mean ± SD and statistical significance determined by p value which is $p < 0.05$.

3. Results

3.1. Blood urea nitrogen levels

Blood urea nitrogen concentrations were found statistically similar in all groups at the start of the study which confirmed that all sheep posed similar metabolic conditions ($p < 0.05$). BUN results were also found significant between different measurement times ($p < 0.001$). Also over the course of the study, interactions between groups and measurements remained insignificant.

3.2. Uterus pH and temperatures

No effect of diet protein or BUN levels on uterus pH and temperature were found at the 7th day of mating ($p > 0.05$). However, as the amount of diet protein increase, pH values were found to increase between groups (7.46; 7.1; 7.06).

3.3. Ovarian responses

Following implementation of hormonal treatment, responses were determined by counting ovarian structures through flushing procedure. Groups 1 and 2 showed similar ovarian activation (luteal and follicle structure numbers are similar: 198 and 194 respectively) however Group 3 has shown significantly lower ovarian activity (146). Fifty-six sheep (89%) were responded to superovulatory treatment by presenting enough number functional CL, with no significant difference between groups. Between nutritional flushing groups for determination of average ovulation, the highest number (8,61 ± 0,62) found in Group 2 and the lowest (6,28 ± 0,66) were observed in Group 3 in which both were statistically insignificant. Enough ovulated sheep rates or in other words, superovulation rates (SOR) (in order of groups respectively; 95%, 100%,

and 90%) were insignificant. Again, super-stimulation rates found respectively in order of groups were 80%, 95%, and 85% and found insignificant.

3.4. Embryo recovery rates

Embryo recovery rates following surgical uterus flushing procedure were, 58.70%, 62.98%, and 77.27% respectively. Group 3 has the highest recovery rate under high protein concentrate condition, which also found to be significant.

3.5. Embryo quality

The quality of embryos (evaluated as good/transferrable and bad non-transferrable) among groups are presented in Table 3. Good quality embryo numbers for each sheep were found the highest in Group 2 (2,71 ± 3,01) and found the lowest in Group 1 (2,14 ± 3,26). Bad quality embryo numbers for each groups were found as 0,38 ± 1,32; 0,85 ± 1,71; 0,66 ± 0,85 respectively. Besides the general aspect of assessing good and bad embryonic quality, determining embryo numbers as a rate helped better understand the effects of variable factors. By evaluating both good and bad quality embryos, good quality embryo rates found as 85%, 76%, and 79% respectively. Numerical values were found relatively higher than rates observed in Group 1. Unfertilized oocyte rates were determined by counting observable CL numbers and found as 25%, 22%, and 26% respectively. There is no correlation between BUN levels and embryonic quality. However, when the comparison of number of good quality embryos to total embryo count concluded, Group 1 has shown the highest rates (85%) and Group 2 has shown the lowest rates (76%).

4. Discussion

The study aimed to investigate effects of protein based flushing related BUN effects on ovarian response, embryo recovery and embryo quality in superovulated native Anatolian sheep breeds during the reproductive season. The present study has shown that nutritional treatment applied prior to and during sexual season modifies both ovarian responses and embryo recovery rates. Nevertheless, variations in concentrated protein rates of flushing diet applied to three groups did not show any significant difference in terms of embryonic quality; uterine physiology (pH and temperature) or to related BUN levels ($p < 0.05$). Mean values for numbers of ovulation following superovulation treatment did not show any significance among flushing groups.

However, evaluation of ovarian responses following superovulation treatment is conducted by counting large and non-ovulated follicles and CL which are considered as feasible and showing statistical significance between groups ($p < 0.05$). In addition, results have shown that supplementation of high protein diet is affecting negatively superovulation responses when compared to lower protein diet. When we look at to the effects of protein rate on superovulation, it was shown that high protein rates decrease superovulation performance related to luteal and follicular structures whereas lower protein diet supplementation found could be related low ovarian response and un-ovulation.

Increasing protein ratio in diets increases BUN levels, also increase concentration of urea nitrogen in different tissues and organs such as ovarian follicles, uterus, and other tissues. This increase affects uterus directly by its lumen as stated by Butler [22]. Various other factors affect BUN levels such as metabolic energy and diet protein type. In this regard Koenig et al. [23] reported that nitrogen retention increases by protein intake, which has related to energy intake in cows. Also found results were similar to previous findings on superovulation treatment applied to cows [24,25]. Similar research has generally conducted on highly efficient dairy cows, which fed to high-protein concentrates and effects on fertility and on some other reproductive performance parameters have been assessed. Nevertheless, there was no extensive study about sheep at the time of this research. Some research has published on dairy cows concluded that by providing high protein diet BUN levels increased yet negatively effects become apparent on fertilization; some reproductive outcome and embryo quality [26–29].

Despite the fact that average serum urea nitrogen level is 8–20 mg/dL in sheep in the clinical perspective, high BUN levels are common in tidy studies and not the indicator only for disease or disorder. Relatively high BUN levels in the present study may be associated with, minimum digestion activity and minimal metabolic activity at the time of blood collection (in the morning) and while also inadequate water and food intake [30]. It is also concluded that there were some factors that affect BUN levels including stress, strain, hydration status and age [31]. Also, alfalfa hay generally used for roughage requirement for feeding has the high binding activity of nitrogen and contains 14% protein as dry mass may be related elevated BUN levels [31]. In supportive experiments, it is also stated that elevated BUN levels show variance throughout the day and depending on the diet change in dairy cows [2,5,31,32].

From a different viewpoint, Dawuda et al. [33] reported that feeding regimes with long term and short term urea supplemented concentrated diets affect BUN levels by increasing significantly three hours following application yet then become stationary thereafter. However some studies conducted by various researchers of which Preston et al. [34] (8–32 mg/dl), Rhoads et al. [35] (15–24 mg/dl), Butler et al. [7] studied in cow (16–29 mg/dl); Kia et al. [36] studied in goat (21–24 mg/dl) and Antunovic et al. [37] (16–19 mg/dl), Piccione et al. [38] (19–35 mg/dl) and Yıldız et al. [39] (5–25 mg/dl) studied in sheep, are all reported relatively low BUN levels when compared to present study conducted on native breeds of Anatolian sheep. In any case there are some reports, Karen et al. [40] (6–76 mg/dl), Dosky et al. [41] (46–66 mg/dl) and

Table 3
Superovulation responses, recovered embryos and cells number on different protein diets.

Treatment (n = 21)	Group 1	Group 2	Group 3
CL numbers	7,38 ± 4,98	8,61 ± 6,46	6,28 ± 2,81
Large Follicles Numbers	2,04 ± 2,59	0,61 ± 1,39	0,66 ± 1,11
Ovarian responses (follicle + CL, $p < 0.05$)	9,42 ± 5,23	9,42 ± 5,23	6,95 ± 2,71
Superovulation rates (%)	95	100	90
Number of cells recovered	4,33 ± 3,83	5,42 ± 4,47	4,85 ± 3,13
Unfertilized oocyte numbers	1,80 ± 2,58	1,85 ± 3,59	1,85 ± 3,59
Embryos recovered	2,52 ± 3,90	3,57 ± 4,26	3,23 ± 3,49
Good quality embryos recovered	2,14 ± 3,26	2,71 ± 3,01	2,57 ± 2,95
Good quality embryos recovered	0,38 ± 1,32	0,85 ± 1,71	0,85 ± 1,71
Recovery rates (%, $p < 0.05$)	58,7	62,98	77,27

Küçükersan et al. [42] (35–50 mg/dl) have presented similar results in sheep.

To put in another point as well as Karen et al. [40] reported that feeding with high crude protein diet would result in a wider range (2–27 mmol/l = 6–76 mg/dl) of plasma urea nitrogen levels without any impairment on breeding and on pregnancy rates. Again, Boland et al. [13] reported that high diet urea and plasma urea concentration level had shown no effects on ovulation rate but resulted in lesser yields of eight blastomere embryos on the 4th day. This might be due to the oviduct environmental changes and follicular impairments.

A different standpoint, McEvoy et al. [43] have reported that urea and urea metabolites were similar for sheep and cows yet there was always a fertility hampering when urea was added to the diet. Also, Butler et al. [7] reported that increased BUN levels (more than 19 mg/dL) due to elevated diet protein is related to decreased pregnancy rates. However, Kia et al. [36] have reported that high protein flushing combined with hormone treatment applied to Iranian Markhoz goats resulted in better reproductive performance compared to low protein diets. While explanations on effects of diet protein rates on BUN levels were given, there were no consistent reports about effects on reproductive performance.

Even though there was no literature on BUN concentration relation with the breed, it might be concluded from the study of Küçükersan et al. [42] on native bred shows that native sheep breeds might show more elevated BUN levels in reaction to higher protein ratio. However, to get a cause effect relationship there is more need for the studies that focused on the topic. In other words, it might be claimed that native breeds have a lesser capacity to use of nitrogen in microbial protein synthesis.

Uterus lumen has a dynamic environment due to its cyclic status and pregnancy period by influences of steroid hormones. In accordance with Butler [10] for a successful embryonic development, there is always a need for a healthy physicochemical intra uterine environment. Also, Barnes [44] stated that synchronized interactions between embryo and uterus environment are important for initiation of pregnancy and maintenance.

Blood urea nitrogen changes are in response to feeding may alter electrolyte balance inside the uterus and therefore the embryonic quality [6]. However, there is no enough study to prove association between uterine lumen pH and feeding regime or BUN concentration, was no correlation was found between BUN levels and pH levels in the present study. Assess to uterus physiology uterine temperature and pH were evaluated. In conjunction with Elrod and Butler [6], heifers, fed to 15% and 22% protein concentrate had shown pH levels of 6.75–6.87 in estrus and 7.09–6.79 in luteal phase respectively. This report is consistent with the present results. The present study also does not show any significant difference between pH measurements and flushing regime.

There is no sufficient information on nutrition about fertility relationship especially in sheep however; it might be stated from the studies that either direct high protein diet or indirect elevation in BUN would decrease fertility. In respect to this Kenny et al. [45] reported that high diet crude protein has a relation to increase in both systemic ammonium and urea concentration and hence it may relate to decreasing fertility. Moreover, Tamminga [46] reported that both ammonium and urea, which are elements of nitrogen cycle, may affect fertility directly both in pre-ovulatory stage and during early embryonic development in cow. Again, Butler [47] stated that high protein diet results in elevated BUN levels and negatively alters uterus environment; in accordance with fertility and some reproductive parameters.

However, no correlation is found between BUN levels and fertility rates in present study in sheep, there are studies [6,10,11,48] indicating that higher BUN and/or milk urea nitrogen (MUN) levels are related to decreased fertility rates in cows.

An embryo recovery rate affected by the expertise of operators and it is a subjective criterion, which may further usually be associated with increased fertilization and decreased ovulation rates [49]. Due to the fact that became reason why groups with higher embryo recovered in numbers had resulted in lower embryo recovery rates in the study. Regardless of how much cells were recovered, it might be evaluated as fertilization rates decreased by some fertilization problems and resulted in fewer embryo recoveries.

There are some reports evaluated, in embryo quality relation with diet protein and urea nitrogen. For instance, Rhoads et al. [35] showed that high plasma urea nitrogen (PUN) levels are attained by high protein nutrition, nevertheless, this does not affect either embryonic quality or embryo recovery rates in cows. More over Lozano et al. [8] stated that nutrition plays an important role in both morphologic and functional qualities on sheep embryos. Excessive feeding may increase embryonic mortality and results in stunted embryonic development. Even though, Abeica et al. [50] reported that deficiency in nutrition would stunt embryonic development and elevates embryonic survival in first two weeks. According to comparisons made while high protein diet is resulted in significantly higher embryo recovery rates, ovarian responses were largely individualistic. Hence Bari et al. [49] reported that both superovulation responses and embryo recovery rates may individually change in the ewe. Again, the highest ovarian response to superovulation treatment; total embryo numbers; the highest good embryo counts and lesser bad embryo numbers found in groups fed at moderate protein levels show that disadvantages of high and low protein diets might be minimalized by medium protein diet.

5. Conclusion

In summary, the present experiment showed that the dietary supplementation in breeding season with different protein grade concentrate influence ovarian responses in some Anatolian sheep breeds. Overall, we might state that low protein diet decreases embryo yields by significantly increasing un-ovulated follicle numbers. We thought that an increase in un-ovulated follicle numbers due to fertilization and/or ovulation failure from low protein diet supplementation might be related to glucose; insulin; leptin and LH concentration which is stated in lots of study.

Finally, data presented here indicate that future investigations concerned with the specific dietary nutrient composition designed to improve sheep reproduction must take into account. Also the probability of several actions of nutrient supply on fertility and reproductive efficiency, from oocyte to embryo quality in different stage of development might be evaluated individually.

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