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Some Biochemical, Cytogenetic and Reproductive Studies Associated with the Use of Hormones and Flushing with Lupine Grains in Sheep

[1]M.M. Bashandy, [2]Dalal S.M. Mostafa and [2]Ghada H.A. Rahman

[1]Department of Clinical Pathology, Cairo University, Giza, Egypt
[2]Biological Reproduction Department, Animal Reproduction Research Institute, Giza, Egypt

Abstract: This study was carried out to investigate the effect of gonadotropin hormones and flushing with lupine grains on body weight, lambs birth weight, some serum biochemical parameters and cytogenetic analysis of forty adult, healthy Barki ewes of 3-5 years with 45-50 Kg. Ewes were divided into four groups (n = 10 each). The first was the control group, the second was treated with PMSG, the third was treated with PMSG and GnRH hormones and the fourth was supplemented with lupine grains. All groups were fed on ordinary diet allover the gestation period. Blood samples were collected monthly all over the experimental period. The results revealed significant increase in ewes' body weight and lambs' birth weight in the supplemented lupine group comparing to the other groups. In the first and fifth month of the experiment, values of total proteins, albumin and glucose, revealed significant decrease in the hormonal treated groups than in the other two groups. There were significant increases ($p<0.05$) in the measured levels of lipid profiles as well as hormonal levels of progesterone and cortisol in the all hormonal treated and flushed groups than the control group. There was a significant increase ($p<0.05$) in the chromosomal aberrations in the hormonal treated groups than that in the control and flushed groups. It was concluded that use of lupine grains can improve reproductive efficiency in ewes and decrease the frequency of chromosomal aberrations and improve the genetic material.

Key words: Sheep · Gonadotropin · Supplementation · Twinning · Chromosomal aberrations

INTRODUCTION

Sheep offer the potential of making an important contribution of providing food and fiber for growing world population. The fact that most ewes in the agriculturally productive countries are seasonal breeders producing smaller lamb crops has made sheep an obvious target for the reproductive physiologists' attention [1]. Reproduction in sheep is influenced by numerous factors e.g genetic potential, nutritional status and environmental factors which are important in both rams and ewes. Because many sheep have the potential for multiple births, we can use managemental practices to influence these factors for increasing their reproductive rate. Short-term nutritional manipulation has resulted in increased ovulation rates following intravaginal sponge ES. [2] Supplementation of ruminants' diets with lupines has been shown to have many positive effects in terms of growth and reproductive efficiency [3] resulting in an increased lambing rate, a shorter breeding season and a decreased number of open ewes.

The present work was a trial to increase the reproductive efficiency using lupine flushing in ewes as well as to compare between the effect of both hormonal treatments and lupine supplementation on the reproductive performance of the experimentally synchronized ewes and assessing the chromosomal aberrations following these treatments.

MATERIALS AND METHODS

Forty adult normally cyclic and clinically healthy Barki ewes (3-5 years of age) were used in this trial. Ewes body weight ranged from 45-50 kg, with body condition scores ranged from 2-3 by feeling the mid lumber region [4]. The current work was conducted in Animal Reproduction Research Institute-Sheep farm and in a private farm at El-Ayyat, Giza governorate. All ewes were

Corresponding Author: Dalal S.M. Mostafa, Department of Biological Reproduction, Animal Reproduction Research Institute, Agriculture research Insitute, Postal code: 12556, Giza, Egypt, E-mail: dalalsaad2005@yahoo.com.

kept under observation for two weeks prior to the experiment to be sure that they are free from any infectious disease and were given prophylactic treatment against internal and external parasites, animals were subcutaneously injected with 1ml/50kg BW ivermectin (Bomectin[R], product of BoMAC Laboratories, 1% W/V). Synchronization of the estrous cycles in all ewes were done using intra vaginal sponge saturated with Medroxy progesterone acetate (Veramix, Upjohn L.t.d. Crawley, Sussex, UK). Ewes were housed under natural lighting condition as one flock. They were divided into four groups in four separate yards during the gestation period till lambing. During gestation period, all ewes were provided with balanced ration, (requirements for maintenance and pregnancy) and water was offered *ad libitum*. Ewes were divided into the following four groups each composed of 10 ewes: Group 1 (The control group) was left without any treatment, the second group (PMSG group) each ewe was treated by I/M injection of 250 iu of Pregnant Mare Serum Gonadotropin (PMSG) once after 13 days from synchronization (Folligon, Intervet, Holland), Group (3) (PMSG and GnRH group) each ewe of this group was treated once with (PMSG) as in group (2) seven days later injected by I/M injection of 500 iu of gonadotropin releasing hormone (Fertagyl, Intervet, Holland) (GnRH) [3], group (4) each ewe was supplemented with 1¼ cup of crushed lupine grains during the first 10 days after synchronization of estrus [3]. After all ewes had exhibited estrus, two rams were introduced to the flock to allow natural mating. Pregnancy was diagnosed and fetal numbers were determined by the mean of Ultrasonography :5-7.5 MHZ linear array transrectal probe with scanner 200 Vet. (Pie Medical Co. Mastricht- Holland) used for detection of number of embryo.

After ten weeks of pregnancy, ewes were collected according to age, weight and number of foeti in the yards until the day of lambing. All the pregnant ewes were kept under observation throughout the gestation period to record any clinical manifestation occurred during the trial. All ewes were weighted and scored at the end of experiment and the percentage of pregnancy, fertility and prolificacy were calculated [6]. Newly born lambs also were weighted immediately after lambing. Blood samples were monthly collected taken from each ewe through jugular vein puncture during the gestation period. The blood sample was divided in to two parts; the first was collected in a clean centrifuge tube for serum separation and kept for biochemical analysis of serum total proteins [7], albumin [8] and glucose [9]. Also, concentrations of lipid profile including; total cholesterol [10], total lipids [11] , triglycerides [12] were determined. The assays of serum progesterone [13] and cortisol [14] were carried out using Radioimmunoassay (RIA). The other part of the blood was aseptically taken into tubes containing free lithium heparin (10 i.u. /ml blood) and was used for the cytogenetic analysis [15] which were carried out. Under hygienic measures on the samples taken during the fifteen days after the end of treatment. Briefly samples were received into sterilized heparinized vacutainers with special care in handling and were kept in 37-38 °C for chromosomal analysis. The slides were scanned under low power for suitable metaphase spread, then 50 good metaphases from each animal were examined microscopically (X100) and all the chromosomal aberrations were recorded [16]). Statistical analysis was done by two ways ANOVA [17].

RESULTS

Results of the reproductive performance (pregnancy percentage, total number of lambs born, number of single lambs born, number of twin lambs born, litter weight at birth, fertility percentage and prolificacy percentage) and the body weight of different experimental groups are shown in tables 1 and 2. The highest pregnancy percentage was recorded in group (4) followed by group (3), group (2) and finally group (1) as it was (100, 90, 80 and 50%, respectively). The highest twin percentage was recorded in the hormonal treated groups than that in the lupine-flushed and the control groups as it recorded 77.7% in the third group, 62.5% [in] the second group, while it was 44% and 0% in the fourth and the first groups respectively.

The mean values for the total proteins, albumin, globulins revealed that there was significant ($p<0.05$) hyperproteinemia and hyperglobulinemia at the 1st and 2nd months of the trial in the fourth group comparing with the other experimental groups, meanwhile there was hypoproteinemia and hypoalbuminemia in groups 2 and group 3 during 4th and 5th months of the trial (Table 3).

The mean values of serum total lipids, total cholesterol and triglycerides significantly ($p<0.05$) increased in the three experimental groups than that in the control one all over the study (Table 4). There was significant ($p<0.05$) hypoglycemia in the two hormonal treated groups in the 4th and 5th month, while there were non-significant changes in group (4) all over the experimental period comparing with the first one (Table 5). The mean values of the progesterone and cortisol hormones showed significant increases ($p<0.05$) in the all hormonal treated and lupine-flushed groups versus the control one all over the experimental study (Table 6).

Table 1: Reproductive performance of different experimental groups

Items	Group (1)	Group (2)	Group (3)	Group (4)
Pregnancy percentage	50% [a]	80% [b]	90% [c]	100% [d]
Total number of lambs born	5 [a]	13 [b]	16 [c]	14 [d]
Single lambs percentage	100% [a]	37.5% [b]	22.2% [c]	60% [d]
Twin lambs percentage	0 % [a]	62.5% [b]	77.7% [c]	40% [d]
Litter weight at birth (kg)	3.1±.14 [a]	4.3±.16 [a]	4.2±.12 [a]	5.01±.05 [b]
Fertility percentage	50% [a]	130% [b]	160% [c]	140% [d]
Prolificacy percentage	100% [a]	162.5% [b]	177.78% [c]	140% [d]

Means with different superscripts (a, b, c, d) within a row are significantly different at p<0.05.

Table 2: Values of body weight (kg/bw) of different experimental groups (mean ± SE)

Month	Group (1)	Group (2)	Group (3)	Group (4)
First	45.71 ± 0.60 [a]	45.98 ± 0.55 [a]	46.77 ± 0.64 [a]	48.36 ± 1.43 [a]
Second	46.01 ± 1.60 [a]	47.3 ± 0.60 [a]	47.9 ± 0.51 [a]	51.50 ± 1.20 [a]
Third	48.30± 0.60 [a]	49.9 ± 0.70 [a]	50.1 ± 0.50[a]	54.7 ± 0.20 [b]
Fourth	47.32 ± 0.50 [a]	51.5 ± 0.12 [a]	52.6 ± 0.71 [a]	57.05 ± 0.12 [b]
Fifth	53.32 ± 0.45 [a]	54.9 ± 0.53 [a]	53.93 ± 1.28 [a]	59.85 ± 1.68 [b]

Means with different superscripts (a, b, c) within a row are significantly different at p<0.05.

Table 3: Values of serum total proteins and albumin in different experimental groups (mean ± SE)

	Total protein (g/dl)				Albumin (g/dl)			
Months	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
First	7.16±0.16 [a]	7.21±0.24 [a]	7.27±0.13 [a]	9.12±0.15 [b]	3.27±0.45 [a]	3.39±0.63 [a]	3.45±0.44 [a]	3.90±0.39 [a]
Second	7.72±0.82 [a]	7.37±0.65 [a]	7.55±0.44 [a]	8.97±0.68 [b]	3.48±0.55 [a]	3.52±0.50[a]	3.58±0.49 [a]	3.59±0.46 [a]
Third	7.14±0.52[a]	7.43±0.71[a]	7.48±0.57[a]	7.86±0.73[a]	3.43±0.36 [a]	3.48±0.42 [a]	3.52±0.38 [a]	3.63±0.51 [a]
Fourth	7.11±0.41[a]	6.43±0.40 [b]	6.57±0.40 [b]	7.96±0.31 [a]	3.27±0.16 [a]	2.49±0.32 [b]	2.71±0.43 [b]	3.56±0.55 [a]
Fifth	7.02±0.28[a]	6.38±0.09 [b]	6.10±0.32 [b]	7.16±0.28 [a]	3.00±0.31 [a]	2.49±0.41 [b]	2.40±0.63 [b]	3.29±0.60 [a]

Means with different superscripts (a, b, c) within a row are significantly different at p<0.05.

Table 4: Values of serum total lipids, cholesterol and triglycerides of different experimental groups (mean ± SE)

	Total lipids (mg/dl)				Cholesterol(mg/dl)				Triglycerides(mg/dl)			
Months	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
First	345.21±7.13[a]	393.65±7.65[b]	392.13±7.82[b]	387.68±7.24[c]	64.61±2.89[a]	83.04±2.91[b]	84.67±1.35[b]	76.67±2.38[c]	50.67±1.07[a]	71.24±2.39[b]	72.27±3.16[b]	67.87±2.64[c]
Second	356.91±3.09[a]	392.55±6.54[b]	392.33±3.92[b]	386.18±5.26[c]	66.51±1.95[a]	82.74±2.83[b]	85.98±1.37[b]	79.47±1.12[c]	51.66±1.46[a]	73.52±4.82[b]	74.07±9.42[b]	70.27±1.68[c]
Third	365.91±4.01[a]	390.75±5.63[b]	393.83±7.93[b]	388.87±3.46[c]	68.41±1.64[a]	84.53±2.05[b]	88.58±2.38[b]	80.77±2.20[c]	53.25±1.01[a]	74.12±5.19[b]	78.87±1.95[b]	66.75±1.53[c]
Fourth	374.74±7.37[a]	393.35±3.45[b]	392.53±4.76[b]	385.68±6.35[c]	70.22±1.43[a]	89.24±2.16[b]	90.08±2.47[b]	82.27±2.61[c]	55.46±3.11[a]	75.02±2.41[b]	79.37±2.33[b]	67.07±1.60[c]
Fifth	368.24±2.31[a]	399.85±4.54[b]	403.73±8.60[b]	389.68±5.86[c]	71.92±1.17[a]	90.14±2.92[b]	92.08±2.15[b]	87.07±2.87[c]	56.47±1.29[a]	76.42±2.91[b]	77.46±1.21[b]	68.92±1.90[c]

Means with different superscripts (a, b, c) within a row are significantly different at p<0.05.

Table 5: Values of serum glucose different experimental groups (mean ± SE)

	Glucose (mg/dl)			
Months	Group 1	Group 2	Group 3	Group 4
First	57.46±0.79[a]	52.66±1.37[a]	53.98±1.27[a]	56.60±1.02[a]
Second	56.88±0.75[a]	53.77±1.16[a]	52.29±0.97[a]	55.38±0.94[a]
Third	56.68±0.67[a]	51.27±1.22[a]	50.58±0.65[a]	53.26±0.61[a]
Fourth	52.48±0.78[a]	46.27±1.45[b]	45.28±0.97[b]	54.42±0.26[a]
Fifth	50.95±0.31[a]	41.67±0.95[b]	44.38±0.65[b]	52.34±0.26[a]

Means with different superscripts (a, b, c) within a row are significantly different at p<0.05.

Table 6: Values of progesterone and cortisol assays of progesterone and cortisol hormones of different experimental groups (mean ± SE)

	Progesterone (ng/ml)				Cortisol (ng/ml)			
Months	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
First	5.59±0.23 [a]	9.59±0.31 [b]	9.22±0.29 [b]	8.68±0.29 [c]	6.41±0.72 [a]	16.88±0.49 [b]	18.78±0.47 [b]	13.84±0.56 [c]
Second	6.29±0.24 [a]	13.19±0.36 [b]	14.10±0.57 [b]	10.81±0.57 [c]	6.71±0.98 [a]	11.88±0.52 [b]	12.11±0.36 [b]	9.58±0.50 [c]
Third	8.21±0.22 [a]	16.19±0.30 [b]	18.41±0.31 [b]	13.79±0.35 [c]	6.91±0.12 [a]	16.58±0.44 [b]	14.07±0.57 [b]	10.64±0.49 [c]
Fourth	6.39±0.24 [a]	18.69±0.27 [b]	19.48±0.22 [b]	15.82±0.34 [c]	7.01±0.14 [a]	19.28±0.59 [b]	18.78±0.42 [b]	14.78±0.41 [c]
Fifth	4.19±0.25 [a]	17.90±0.28 [b]	20.14±0.23 [b]	16.84±.28 [c]	8.01±0.18 [a]	25.52±0.54 [b]	27.48±0.50 [b]	20.38±0.46 [c]

Means with different superscripts (a, b, c) within a row are significantly different at p<0.05.

Table 7: Frequency of numerical chromosomal aberrations of different experimental groups.

Groups	Total examined metaphases	Polyploidy		Peridiploidy		Total	
		No.	%	No.	%	No.	%
Group (1)	500	11	2.2	16	3.2	27	5.4
Group(2)	500	24	4.8*	108	21.6*	132	26.4*
Group(3)	500	32	6.4*	119	23.8*	151	30.2*
Group(4)	500	9.0	1.8	15	3.0	24	4.8

* Significant from control at p<0.05

Table 8: Frequency of structural chromosomal aberrations of different experimental groups.

Groups	Total examined metaphases	Gap		Break		deletion		fragment		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Group (1)	500	4.0	0.8	5.0	1.0	8.0	1.6	4.0	0.8	21	4.2
Group (2)	500	15	3.0*	15	3.0*	14	2.8*	15	3.0*	59	11.8*
Group (3)	500	14	2.9*	17	3.1*	20	3.8*	16	3.4*	67	13.5*
Group (4)	500	2.0	0.4	6.0	1.3	2.0	0.4*	3.0	0.6	13	2.7*

* Significant from control at p<0.05

Table 9: Frequency of total chromosomal aberrations of different experimental groups.

Groups	Total examined metaphases	T.N.	T.S.	Total no. of aberrant cells	Percentage of aberrant cells from total examined (%)
Group (G1)	500	27	21	48	9.6
Group (G2)	500	132	59	191	36.8*
Group (G3)	500	151	67	218	43.7*
Group (G4)	500	24	13	37	7.8*

T.N. : Total numerical aberrations. T. S. : Total structural aberrations

* Significant from control at p<0.05

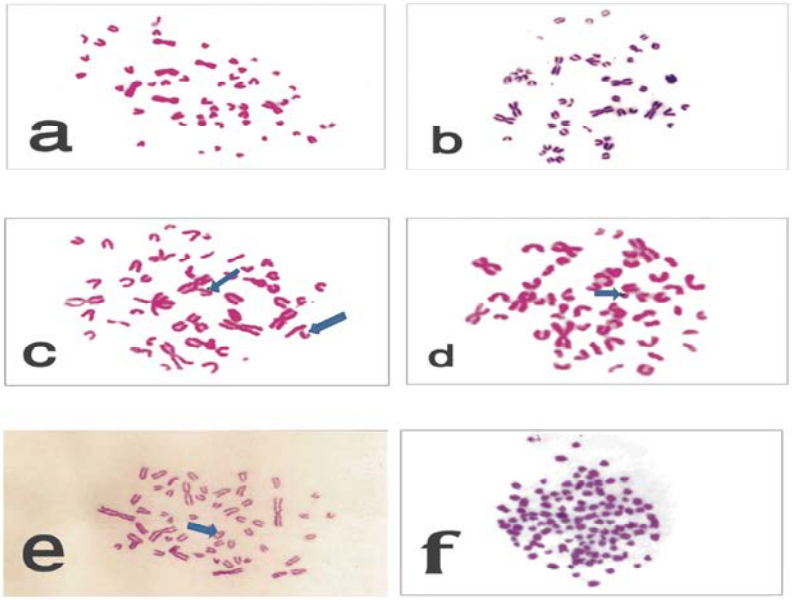

Plate 1: Chromosomal aberrations in different experimental groups. X 100 (Giemsa Stain)

(a): A metaphase spread showing a normal cell.

(b): A metaphase spread showing periploidy.

(c): A metaphase spread showing deletion and gap.

(d): A metaphase spread showing a fragment.

(e): A metaphase spread showing a break.

(f): A metaphase spread showing polyploidy.

The obtained data for cytogenetic analysis (Tables 7-9 and Plate 1) revealed a significant increase ($p<0.05$) in the percentage of total chromosomal aberrations in both the two hormonal treated groups (36.8, 43.7 and 9.6%) for the second, third and the first groups respectively). On the other hand lupine flushed group showed a lower frequency of total chromosomal aberrations (7.8%). There was a significant increase ($p<0.05$) in structural aberrations in the second and third groups (11.8 and 13.5%. respectively) in comparison to control group (4.2%). The observed structural aberrations were gaps, breaks, deletions and fragments. Deletions aberrations revealed a significant increase ($p<0.05$) in the second and third groups (2.8 and 3.8%. respectively) while the fourth group showed a lower frequency of deletion (0.4%) in comparable to the control group (1.6%).The percentage of aberrant cells with fragments was (3.0 and 3.4%) in the second and the third groups respectively. Lupine flushed group recorded a lower percentage (0.6%) as compared to the control group (0.8%). Breaks recorded a significant increase ($p<0.05$) in both the second and the third groups (3.1 and 3.0%. respectively) while the lupine flushed group recorded a lower percentage (1.3%) as compared with the control group (1.0%). The frequency of aberrant cells with gap was significant ($p<0.05$) increase in both the second and the third groups (2.9 and 3.0%. respectively) versus the control group (0.8%), whereas lupine flushed group showed a lower frequency (0.4%). The observed numerical aberrations were classified into polyploidy and peridiploidy (2n-1 and 2n-2). The percentage of aberrant cells with numerical aberrations showed a significant ($p<0.05$) increase in both the second and the third groups (30.2 and 26.40%. respectively). On the other hand, lupine flushed group showed a lower frequency of total numerical aberrations as they were (4.8%) in comparison with the control one (5.4%). The frequency of aberrant cells with polyploidy were (6.4 and 4.8%) in the second and the third groups respectively, while lupine flushed ewes showed a lower frequency of ploypoidy (1.8%) versus the control one (2.2%). The percentage of peridiploidy recorded a significant ($p<0.05$) increase in both the second and the third groups (21.6 and 23.8%. respectively) while the lupine flushed group revealed a lower percentage (3.0%).

DISCUSSION

In Egypt, sheep are widely spread animals and considered as an important source of meat, milk and wool, in addition to its high fertility and has short reproductive period. The present study discussed and interested to determine whether flushing short term manipulation lupine with high protein and energy content as well as the gondadotropin hormones treatment are recommended for ewes reproduction or not. In the present study the pregnancy percentage was the highest (100%) in the lupine flushed group followed by the hormonal treated groups. Average number of lambs born per ewe was significantly increased in the hormonal treated groups. The percentage of ewes lambed twins was significantly increased in the hormonal treated and flushed groups versus the control one, at the same time group (3) had highest percentage (77.7%). Litter weight at birth and body weight of pregnant ewes were increased significantly in lupine flushed group. Gonadotropin hormones commonly used for super ovulation are PMSG or FSH of porcine or ovine origin [18]. The half life of PMSG greatly exceeds than of FSH. This long half life of PMSG induces extra follicular growth during the first follicular wave post ovulation with a resultant increased oestradiol concentration in the blood, which may have a deleterious effect on early embryonic development [19]. However, PMSG was chosen to induce super ovulation in sheep of the current study because of its availability, relatively low cost and easy application with only a single injection. Concerning the influence of GnRH on the ovarian response and production of birth lambs, the results of the study revealed that, the reproductive performance of the third group [PMSG and GnRH] was significantly increased in comparison with the group (2) [PMSG]. These results agreed with the results of Thibier *et al*. [20], who reported a significantly higher number of embryos in GnRH treated cows than that of the control group. Similarly, the administration of GnRH resulted in a higher fertility rate and recovery of more developed embryos, the higher rate of twining and litter size in the GnRH treated ewes in comparison to their control ones. The mechanisms involved in the different regulation of LH and FSH by GnRH treated remain unclear [21]. Another important function of GnRH is to elicit sexual receptivity, thus the onset of the preovulatory LH surge and sexual receptivity are coordinated via GnRH synthesis and release [22]. The administration of PMSG and GnRH to local ewes increased the number of lambs born per ewes as the direct reflect to induction of multiple ovulations. These results are in agreement with those reported by Ibrahim [23]. The high performance of lupine flushed group may be due to the composition of lupine which contain 28 to 48 % crude protein, FSH, glucose, insuline, leptin, growth hormone (GH) and insulin like growth factor

(IGF-I) which reduce the rate of corpus luteum atresia and increasing the number of follicles that develop to the ovulatory stage. It has been suggested that IGF-I can reach the ovine ovary through a local pathway, which could then act in an endocrine manner to affect follicular cell development. Moreover, the short term supplementation with lupine grains increased glucose, insulin and leptin and this may increase the capacity of the follicles to utilize FSH [24]. Similar results were reported by Hossain *et al*. [25] and Viñoles[26].

Regarding to the protein profile, it showed significant decrease in the hormonal treated groups on the 4th and 5th month. Lupine flushed group showed significant increase with at the 1st and 2nd months. Significant decrease in the total proteins, in the hormonal treated groups may be due to the hypoproteinemia occurred naturally in ewes carrying twin lambs during the late months of pregnancy and due to great nitrogen consumption by the foeti [27] . Also, Feldman *et al.* [28] recorded that plasma proteins vary greatly in their amino acid composition and nutritional adequacy during pregnancy, while, El-Sayed [29] recorded hyperprotienamia as a result of elevation in cortisol level leading to increase amino acids concentrations. Hyperglobulinemia in the lupine flushed group in the first months of the gestation may have been occurred as a result of the sensitization of immune response under the effect of growth hormones stimulation as mentioned by Taha [30] who found improvement in immunoglobulin G (IgG) level as a response to growth hormone effect or high level of (IgG) in the contents of lupine legumes or due to increase in humeral antibody due to growth hormone. Furthermore, it was found that the plasma protein concentration increased after GH treatment [31] and continued in its increase through out the trial. On contrary, in most cases, non significant differences in the investigated parameters of protein were detected among groups of animals fed lupine diets [32], while Singh *et al.* [33] reported a decrease in serum total proteins.

Results of lipid profiles showed increases in all the experimental groups versus the control one all over the experimental period, the explanation of this elevation in serum may be due to stress of twining which led to elevation in steroidogenesis which is initiated by binding of ACTH to high affinity receptors in the adrenocortical membrane resulting in the activation of adenylate cyclase which then result in increasing intracellular cAMP. The cAMP activates phosphoprotein kinases which phosphorylate proteins that increase the conversion of cholesterol estres to free cholesterol [34]. Moreover ACTH stimulates lipolysis in adipose tissue and isolated fat cells. Specific ACTH receptors have been reported to be present on fat cells. This action of ACTH appears to be calcium dependant and is associated with increase in adipose adenylate cyclase activity and cholesterol formation [35]. Similar results of lipogram were recorded by Stockham and Scott [36].

In the present work, the hormonal treated groups showed significant hypoglycemia in the late gestation period, while non significant changes were noticed in lupine flushed group all over the experimental period. The observed hypoglycemia may be due to the high fetal demand for glucose as the placenta can transport glucose from maternal to foetal plasma, so when the imbalance occurs between the maternal ability to synthesis or absorb glucose and fetal consumption, hypoglycemia occurs [22] similar results were recorded by Conway *et al.* [37] and West [38]. Furthermore, in the present study, the significant increase of progesterone level noticed in the hormonal treated and the flushed groups may be due to the high twining percentage as the progesterone concentration released from Medroxy progesterone acetate vaginal sponge as well as to the progesterone secreted from corpus luteum during the gestation period [39]. In addition, injection of hormones enhanced follicular growth [40, 41] and increased LH response to GnRH and increased serum progesterone [42]. Moreover, flushing with lupine grains release insulin like growth factor (IGF-I) which consider as an important potentiator and mediator of the effects of FSH on the ovary so increase the size and number of corpus luteum leading to increase progesterone concentration [43]. Also, lupine is rich in (GH) increased the weight of corpus luteum and increased the plasma progesterone concentrations [44]. The progesterone of pregnant ewes are correlated with the number of corpora leutea or the number of foeti. These results were in agreement with the results reported by Kadzere *et al.* [45]. The increase of cortisol levels in the hormonal treated and the flushed groups may be due to the Medroxy progesterone acetate vaginal sponge treatment effect on the adrenocortical function and the pregnancy especially in twins which considered as stress and stimulates the secretion of corticotrophin releasing factor (CRF) from the hypothalamus, which signals the pituitary to release adrenocorticotrophic (ACTH) from the pituitary, the end product of the action of adrenocorticotrophin is the cortisol [46, 47]. The increase in the cortisol level may be due to the increase in progesterone level and may contribute in the increase of cortisol level as progesterone is considered as a precursor of cortisol [46, 47]. These results were in agreement with the results reported by Stockham and Scott [48].

Regarding cytogenetic analysis, our study revealed that PMSG or GnRH exert a mutagenic effects as they induced significant increases in the percentage of chromosomal aberrations in comparable to the control group. On the other hand lupine supplemented group showed a significant decrease in the percentage of chromosomal aberrations as compared with the control group. The observed chromosomal aberrations were in the form of structural and numerical aberrations. There was a significant increase in the frequency of structural aberrations (breaks, gap, deletions and fragments) in the hormonal treated groups versus the control one. The obtained structural chromosomal aberrations may be attributed to the interactions between endogenous and exogenous hormones in superovulated animals [49]. Our results are comparable to that reported by Fujimoto *et al.* [50] and Kandil *et al.* [51]. The observed numerical chromosomal aberrations were in the form of polydiploidy and peridiploidy. The hormonal treated groups showed a significant increase in the frequency of aberrant cells with numerical aberrations in comparable the control one. The obtained numerical chromosomal aberrations may be attributed to the detrimental effect of super ovulation on chromosomal complements [52]. Moreover, PMSG may interact with DNA during cell division exerting a mutagenic effect [53]. Contrary to Fechheimer and Beatty [54]; who concluded that the super ovulation with follicle stimulating hormone (FSH) and leutinizing hormone (LH) has no demonstrable effect on the incidence of heteroploidy in rabbits, this difference may be attributed to the hormonal preparation or it may be due to species difference [55]. The lupine supplemented group showed fewer incidences of chromosomal aberrations comparing to the other groups. Lupine seeds contain quiolizidinic alkaloids which showed antimutagenic effect [56]. Enrique *et al.* [57] found that the protein content of the whole seed of sweet lupines ranged from 34 to 44% and much higher than those in the common grain legumes. The protective role of protein containing diet was observed by many investigators. A lower incidence of tumors has been correlated with high legume and cereal consumption [58]. Moreover, lupine is considered as excellent source of genistein and diadzein which have a potent antioxidants and antimutageni.

From the results of the present study, it can be concluded that use of lupine grains can improve reproductive efficiency in ewes through improving fertility and prolificacy, improving general health condition and weight, increasing lambs, birth weight, decrease of the frequency of chromosomal aberrations and improvement genetic material.

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