The influence of a slow-release multi-trace element ruminal bolus on trace element status, number of ovarian follicles and pregnancy outcomes in synchronized Afshari ewes

Abdollahi, E.[1]; Kohram, H.[2*]; Shahir, M. H.[3] and Nemati, M. H.[1]

[1]Graduated from College of Agriculture, Zanjan University, Zanjan, Iran; [2]Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran, and Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [3]Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

[*]***Correspondence:*** *H. Kohram, Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. E-mail: kohram@can.ut.ac.ir*

(Received 20 Jan 2014; revised version 23 Jul 2014; accepted 26 Jul 2014)

Summary

Published data on the effects of ruminal bolus on the number of ovulatory follicles in ewes does not exist. The present study determined the effects of a ruminal bolus on trace element status, follicular dynamics and reproductive performance in ewes. Eighty Afshari cycling ewes were synchronized during breeding season using CIDR for 14 days and assigned to 4 groups (n=20); group 1 received a single Ferrobloc bolus four weeks prior to CIDR insertion following 400 IU eCG on CIDR removal, group 2 received two boluses four weeks prior to CIDR insertion following 400 IU eCG on CIDR removal, group 3 received only 400 IU eCG on CIDR removal and group 4 (control) received no bolus and no eCG. Transrectal ultrasonography was done to monitor the ovarian follicles on the day of CIDR removal and a day later. Results showed that boluses increased the status of copper, selenium and iodine on mating day and days 90 to 100 of gestation. Ruminal bolus did not significantly increase the number of different classes of ovarian follicles in ewes fed a diet meeting all trace mineral requirements. All ewes eventually became pregnant with 1 or 2 boluses but the multiple births rate (80%) was higher ($P<0.05$) after 2 boluses compared to the other groups.

Key words: Reproductive performance, Ruminal bolus, Follicle, Trace elements, Afshari ewe

Introduction

Synchronization programs using CIDR insertion for 14 days are commonly used for reproductive management of ewes during breeding season (Barrett *et al*., 2004). Using eCG injections during breeding season for high lambing and a low barren rates is also proposed (Koyuncu and Ozis Alticekic, 2010). However, there are reports indicating that reproductive performance did not increase following CIDR synchronization and eCG injection (Menchaca and Rubianes, 2004). These variable responses may be caused by different study conditions such as nutritional and hormonal statuses of the animals during the synchronization programme (Shipley *et al*., 2007).

Mineral supplementation level is another important factor influencing reproduction in ruminants (Underwood and Suttle, 1999). Mineral deficiencies in the diet or in the uptake may decline ovulation rate, but this is an indirect effect of a primary influence on basic health status (Upadhyay *et al*., 2006). Lamb *et al*. (2008) reported that Heifers receiving trace mineral supplementation for 23 days before embryo collection did not indicate any increase in follicle size and number of ovulated follicles. Another study demonstrated that sub-clinical cobalt/vitamin B12 deficiency around mating time might reduce ovulatory responses in superovulated ewes (Mitchell *et al*., 2007). Nevertheless, no experiments have been performed on sheep to investigate the effect of multi-trace element ruminal bolus on the number of ovulatory follicles.

Adequate trace mineral intake and absorption are needed for growth physiology and reproduction. For example, in ruminants, maternal copper deficiency may cause infertility, delayed or suppressed estrus and decreased conception rate (Underwood and Suttle, 1999). Manganese deficiency increases infertility, abortion, silent estrus and anoestrus in cattle and ewes (Upadhyay *et al*., 2006). Selenium deficiency is reported to lead to silent estrus, early embryonic death, still birth or weak offspring and abortions in cattle (Upadhyay *et al*., 2006). Cobalt/vitamin B12 status during pregnancy has also been shown to affect the lamb viability at birth (Mitchell *et al*., 2007). Scales (1974) reported that the proportion of barren ewes was reduced by orally administering 5 mg Se as sodium selenite or as a powdered selenium/iron supplement before mating. The proportion of twins was also found to increase through the supplementation of copper/selenium (Hill *et al*., 1969) or by mineral and vitamin boluses (Hemingway *et al*., 2001).

The authors of the present work suggest that for ewes which are given minerals before the synchronization protocol, element status and number of ovarian follicles might increase, resulting in the improvement of pregnancy chances and maintenance. Therefore, the objective of this study is to evaluate the effects of sustained-release, multi-trace element ruminal bolus supplementation on trace element status, the average number of ovarian follicles and reproductive performance in terms of pregnancy and multiple births rate following the synchronization protocol in ewes during breeding season.

Materials and Methods

The experiment was conducted during breeding season (September-April), at the Zanjan University farm located in Zanjan city. The site is situated at 48.31 ± 21 °E longitude and 36.40 ± 13 °E latitude and at an altitude of 1663 m above sea level. The mean annual temperature is 14°C and the annual rainfall ranges from 350 to 380 mm.

Animals and synchronization protocol

Eighty 3-4-year old cycling multiparous fat-tailed Iranian Afshari ewes, weighing 65.2 ± 1.8 kg, were used in the trial. All ewes were fed a constant diet of a alfalfa hay [948.9 (g/kg) DM, 123.6 (g/kg DM) CP, 351.6 (g/kg DM) CF], 3.0 (g/kg DM) minerals, 0.4 (g/kg DM) vitamin A, D and E premixes and concentrate feed [crushed corn and soybean meal] [946 (g/kg) DM, 12.4 (MJ/kg DM) ME, 158.8 (g/kg DM) CP] a month before the trial. The diet was formulated to be adequate in protein, energy, vitamin and mineral content to secure the intake of nutrients required for maintenance in accordance with the NRC (2007). 0.5 kg/animal of the concentrate was distributed twice a day (morning and evening) in addition to 2.0 kg alfalfa hay. Water was available *ad libitum* in the shed. Animals were synchronized using CIDR (EAZI-BREED™, CIDR®, New Zealand), for 14 days and assigned into 4 groups. Group 1 (n=20) received a single Ferrobloc bolus (Laprovet, Town, France - a mineral mixture containing 6.9% Ca, 0.711% Mg, 0.312% Na, 0.333 g Cu, 0.16 g Mn, 0.024 g I, 0.396 g Fe, 0.06 g Co, 0.036 g Zn, 0.008 g Se) four weeks prior to CIDR insertion in rumen following an intramuscular injection (i.m.) of 400 IU eCG (Pregnecol, Bioniche, New Zealand) on CIDR removal (day 0). Group 2 (n=20) received two Ferrobloc boluses four weeks prior to CIDR insertion in rumen following an intramuscular injection (i.m.) of 400 IU eCG on CIDR removal (day 0). Group 3 (n=20) only received an intramuscular injection (i.m.) of 400 IU eCG on CIDR removal (day 0), and group 4 (n=20; control) received no bolus and no intramuscular injection of eCG. The release period of Ferrobloc boluses is approximately six months. Evaluations of bolus matrix release rates have been made in slaughtered ewes (Hemingway *et al*., 2001). About half of the matrix weight is released in the first two months. Thereafter, daily release rates reduce and each day the mean amounts erode from two months to about six months. All ewes in estrus were mated twice daily (morning and evening in a ram:ewe ratio of 1:4) with rams of similar age and breeding, whose semen was tested before placement (mating). The reproductive parameters in terms of oestrus response (number of ewes showing oestrus/total treated ewes in each group × 100), pregnancy rate (number of total pregnant ewes/number of total ewes in each group × 100), single births rate (number of ewes lambing single/total number of lambing ewes in each group × 100) and multiple births rate (number of ewes lambing twins or triplets/total number of lambing ewes in each group × 100) were recorded (Sirjani *et al*., 2012).

Ultrasonography evaluations

Ovarian follicular activity was monitored by transrectal ultrasonography (Piemedical, Falco100; Holland, 8 MHz), at CIDR removal (day 0) and one day later (day 1). Ultrasonography was performed in the morning when ewes were off fed before examination. Ultrasonographic scans of both ovaries were recorded using an MP4 player (Marshal X720, China). During the ultrasonographic evaluations, ewes were kept in a darkened room and restrained in a fostering crate in standing position. The ovaries were scanned in several planes to identify all visible follicles (>1 mm in diameter; Fig 1). All follicles >1 mm were counted and classified according to their diameter in one of the following classes: small (≤2 mm), medium (>2 to <4 mm) and large (≥4 mm) follicles (Salehi *et al*., 2010).

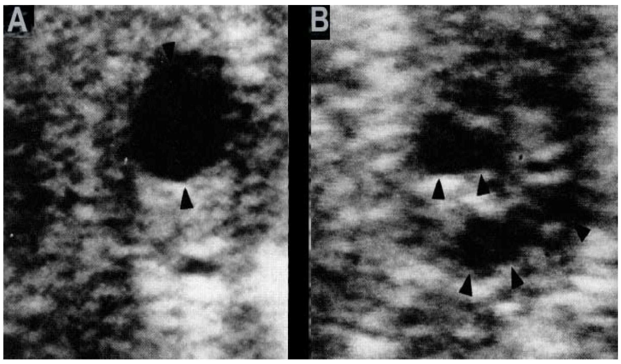

Fig. 1: Ultrasonographic images of the ewes' ovaries obtained using a rigid 8 MHz transducer displayed using a B-mode scanner. (A) A follicle of 6 mm diameter in the ovary, and (B) A group of 1-3 mm follicles

Plasma and assay procedures

To determine plasma trace elements, blood samples were collected in vacuum tubes including EDTA (Venoject®, Sterile Terumo Europe, Leuven, Belgium) from the jugular vein immediately prior to the ruminal bolus treatment, mating time and after approximately 4.5 months (90-100 of pregnancy). Samples were placed on ice 5 h before being centrifuged at 2000 × g for 15 min. Plasma was then transferred to acidwashed storage vials and stored at -20°C. Plasma mineral concentrations were measured after the samples were thawed at room temperature for 3-4 h, and were then treated with Trichloroacetic acid to precipitate the protein. Samples were analyzed for Zn, Cu, Mn, Fe, Se and I using a flame atomic absorption spectrophotometer (Spectra AA20, Varian Co., Australia, Switzerland).

Statistical analysis

Mean plasma concentration profiles of the trace elements (i.e. Zn, Cu, Mn, Fe, Se, and I) were analyzed by carrying out an ANOVA, using prior to treatment profiles a covariate using the general linear model (GLM) procedure of SAS software (SAS, 1996). Least significant differences were used to determine statistical significance between individual group means.

The number of ovarian follicles (i.e. small, medium, and large) was analyzed using the mixed procedure of SAS (1996). Mean comparisons were performed by least square means. The analysis included sources of variation caused by the treatments, days (repeated measures) and their interactions. Differences were compared by a Tukey test. Weight and age of ewes were added as covariates to the model. The frequency of each observation (pregnancy rate, single, and multiple births rate) in the different groups was compared using a Chi-square test. Differences between means were compared statistically at 1% and 5% level of significance.

Results

The sheep given two boluses had significantly higher plasma copper, iodine and selenium at both mating time and on days 90 to 100 of gestation than the control group. Compared to the control group, the eCG + 2 boluses group had an increased iron status at the second blood sampling (Table 1). Plasma manganese and iron concentrations were not significantly raised at 90 to 100 days of gestation. Also, in the eCG + 2 boluses group, plasma zinc concentrations were not significantly higher at mating time compared to the others.

The frequency of each follicle size (small, medium and large) following estrous synchronization is presented in Table 2. The number of small and medium follicles between day 0 and 1 were not different (P>0.05). The largest number of small follicles (P<0.05) in the eCG group was recorded on day 1. The ewes in the 2 boluses + eCG group had more large follicles on day 1 compared to day 0 (3.5 vs 1.7, P<0.05). All ewes following the synchronization protocol and eCG injection had in average ≥3 medium and ≥2 large sized follicles one day before ovulation (day 1).

Values for reproductive parameters are presented in Table 3. Following eCG injection and boluses during the breeding season, all ewes exhibited overt signs of oestrus up to 48 h after CIDR removal. Pregnancy rates in the groups with 2 boluses + eCG and 1 bolus + eCG (100%) were higher than the other groups; however, this difference was not significant (P>0.05). The highest multiple births rate (80%) was observed in ewes that were given 2 boluses (P<0.05) four weeks before estrous synchronization.

Discussion

Compared to the control group, sheep that received two boluses had significantly increased copper, iodine, and selenium status both at mating and days 90 to 100 after treatment; however, plasma zinc concentrations were not significantly higher than others at mating time. According to Kendall *et al*. (2001), when zinc supplementation is given alone, a decrease in copper status could occur due to an interaction between copper and zinc. However, the zinc concentration needed to reduce copper status is generally higher than that released from the bolus (Bremner *et al*., 1976). In the current study, plasma Mn concentrations did not differ between groups at either mating time or days 90 to 100. It has been reported that liver Mn concentration does not reply substantially to Mn supplementation, even at high

Table 1: The effect of slow-release multi-trace element ruminal bolus on trace element status Afshari ewes (pre-treatment values are included for reference)

Item	Groups (n= 20 ewes/group)				SEM	P-value, significance	Pre-treatment values
	1 bolus + eCG	2 boluses + eCG	eCG	Control			
Cu (µg/dl)							138.1
Mating	142.6^{ab}	145.7^{a}	141.0^{ab}	140.3^{b}	1.6	0.04^{*}	
d 90-100	145.8^{b}	155.6^{a}	139.6^{c}	137.8^{c}	2.1	0.00^{**}	
Zn (µg/dl)							98.9
Mating	104.5	105.7	103.5	103.2	1.6	0.71, NS	
d 90-100	107.9^{ab}	114.9^{a}	103.4^{b}	101.1^{b}	2.5	0.00^{**}	
Fe (µg/dl)							189.9
Mating	191.8^{ab}	200.6^{a}	187.9^{b}	187.6^{b}	3.3	0.02^{*}	
d 90-100	192.4	192.9	191.4	189.3	2.2	0.68, NS	
Mn (µg/dl)							115.1
Mating	116.9	118.2	114.9	116.2	1.7	0.58, NS	
d 90-100	121.9	123.2	120.9	121.2	1.0	0.41, NS	
Se (µg/dl)							6.5
Mating	7.9^{b}	10.7^{a}	7.3^{b}	8.0^{b}	0.8	0.03^{*}	
d 90-100	11.2^{b}	15.9^{a}	11.0^{b}	9.2^{c}	0.6	0.00^{**}	
I (µg/dl)							7.8
Mating	10.9^{a}	12.2^{a}	8.3^{b}	7.8^{b}	0.5	0.00^{**}	
d 90-100	12.5^{b}	14.8^{a}	8.9^{c}	8.7^{c}	0.5	0.00^{**}	

NS: Non-significant. * P<0.05, and ** P<0.01

Table 2: Means of small, medium and large follicles on CIDR removal (day 0) and day 1 in different groups of Afshari ewes

Item	Groups (n= 20 ewes/group)				SEM	P-value, significance
	1 bolus + eCG	2 boluses + eCG	eCG	Control		
No. of small follicles						
Day 0						
Right ovary	1.5	1.1	1.4	1.2	0.3	0.43, NS
Left ovary	1.6	1.2	1.5	1.3	0.3	0.41, NS
Total	3.2	2.3	3.0	2.6	0.5	0.35, NS
Day 1						
Right ovary	1.0^{ab}	0.8^{b}	1.6^{a}	0.6^{b}	0.3	0.05^{*}
Left ovary	1.0	0.7	0.9	0.7	0.3	0.42, NS
Total	2.1^{b}	2.2^{b}	3.6^{a}	1.3^{b}	0.5	0.04^{*}
No. of medium follicles						
Day 0						
Right ovary	2.2	2.3	2.8	1.5	0.4	0.53, NS
Left ovary	2.0	1.1	1.6	1.2	0.4	0.43, NS
Total	4.3	3.3	4.5	2.8	0.7	0.46, NS
Day 1						
Right ovary	2.6	1.8	2.3	2.1	0.4	0.39, NS
Left ovary	3.1^{a}	2.4^{ab}	1.6^{b}	1.4^{b}	0.4	0.04^{*}
Total	5.7	4.3	4.0	3.5	0.7	0.45, NS
No. of large follicles						
Day 0						
Right ovary	1.1	0.7^{c}	1.0	0.7	0.6	0.23, NS
Left ovary	1.3	0.9^{c}	1.1	1.0	0.6	0.25, NS
Total	2.3	1.7^{e}	2.2	1.7	0.6	0.21, NS
Day 1						
Right ovary	1.2	1.4^{d}	1.1	0.9	0.6	0.33, NS
Left ovary	1.3	2.1^{d}	1.5	0.8	0.6	0.35, NS
Total	2.4	3.5^{f}	2.6	1.9	0.6	0.10, NS

NS: Non-significant. * P<0.05. Different superscripts ($^{a,\ b}$) in the same row in follicular classes indicate a significant difference. Different superscripts ($^{c,\ d}$) in the same column in follicular classes indicate a significant difference (P<0.05). Different superscripts ($^{e,\ f}$) between totals in the same column and follicular classes indicate a significant difference (P<0.05)

Table 3: Effects of Ferrobloc bolus on the pregnancy and multiple births rate in Afshari ewes

Reproductive traits	Groups (n= 20 ewes/group)			Control
	1 bolus + eCG	2 boluses + eCG	eCG	
Oestrous response % (n)	100 (20/20)	100 (20/20)	90 (18/20)	80 (16/20)
Pregnancy rate % (n)	100 (20/20)	100 (20/20)	90 (18/20)	80 (16/20)
Single births rate % (n)	70 (14/20)^{a}	20 (4/20)^{b}	66.66 (12/18)^{a}	75 (12/16)^{a}
Multiple births rate % (n)	30 (6/20)^{b}	80 (16/20)^{a}	33.33 (6/18)^{b}	25 (4/16)^{b}

Different superscripts ($^{a,\ b}$) between groups in the same row indicate a significant difference (P<0.05)

dietary concentrations (Underwood and Suttle, 1999). Trace element bolus has previously been indicated to cause increased humoral immune response in ewes (Kendall *et al*., 2001). Increases in some trace elements such as copper, iodine and selenium are probable reactions to high immunocompetence, particularly if marginal deficiencies are averted (Kendall *et al*., 2001). Added selenium and iodine supplementation to the sheep's diet has been reported to increase immune responses and vigour and survival rates of fetus (MAFF, 2000; Kendall *et al*., 2001). The release period of Ferrobloc boluses (approximately six months) means that essential trace elements such as copper, iodine and selenium can be supplied when mineral supplementation is required (McDowell, 1992).

Bioavailability governs the absorption and utilization of a nutrient. Results of studies on the bioavailability of minerals such as Cu and Zn from gut absorption, plasma Cu and Zn levels and Cu and Zn contents in the liver suggest that these factors can be used to determine the bioavailability of Cu and Zn (Ammerman *et al*., 1998). Higher phytate and fibre contents in the diet are known to reduce the bioavailability of trace minerals by interacting negatively. In the present study, the ewes were fed with corn (which contains high phytate) and alfalfa hay as a source of fibre, hence creating the possibility of mineral interactions (particularly between Zn and phytate when supplemented through the introduction of bolus to the digestive tract) and resulting in the reduced bioavailability of Cu and Zn.

No reports exist on the effects of ruminal boluses on the number of ovulatory follicles in ewes; nevertheless, the effects of mineral supplementation on ovarian activities and follicular development of dairy cattle (Hackbart *et al*., 2010) and heifers (Story *et al*., 1999; Lamb *et al*., 2008) have been addressed, suggesting that mineral supplementation does not increase the number and size of follicles. In agreement with the above findings, the results of current study showed that giving mineral supplementation (bolus) to ewes had no effect on the number of large follicles one day after CIDR removal in all groups.

Follicular development can be influenced by factors such as nutrition (high energy and high protein) mediated by direct action at the ovary level, involving insulin, peripheral IGF I and intrafollicular IGF-I, leading to follicular development and folliculogenesis (Lamb *et al*., 2008). In the present study, all ewes were given a moderate energy and protein diet that did not exceed NRC (2007) recommendations. The basic difference between treatments was the bolus. It is possible that ewes that were given a diet which met the recommendations for all nutrients negated the potential positive effects of being fed with a more bioavailable mineral such as bolus. Since it was hypothesized that bolus administration enhanced follicle numbers and size, ultrasonographic examinations were done to monitor follicle development. Conversely, no differences were detected in follicle numbers and sizes, demonstrating that bolus(es) did not influence follicle growth four weeks before the start of experiment, which in turn, led us to believe that this period was probably not long enough to affect the ovaries and cause follicular development.

Previous works suggest that trace element deficiency is associated with reduced fertility and litter size and might increase embryo mortality or reduce ovulation rate (Parker and McCutcheon, 1989; Fisher and MacPherson, 1991). Symptoms consistent with low iodine levels, particularly bareness and low reproductive performance in ewes, are being increasingly reported by veterinary practitioners who also report good responses to iodine supplementation (MAFF, 2000). Supplementing ewes with iodine before mating and throughout pregnancy reportedly reduced the numbers of barren ewes per 100 ewes mated (McGowan, 1983). Hartly and Grant (1961) reported the cause of low fertility in New Zealand ewes to be embryonic death at about 3-4 weeks post-conception. Administering selenium along with vitamin E before mating was found to reduce barrenness. Scales (1974) also reported a significant 12% reduction in the proportion of barren ewes in three of four New Zealand trials resulting from the oral administration of 5 mg Se as sodium selenite 17 days before mating. Similarly, in the present study, ewes given bolus(es) became pregnant during the breeding season, suggesting that rumen bolus improved reproductive performance in terms of fertility.

It has been previously reported (Hartley and Grant, 1961; Hemingway *et al*., 2001) that different forms of mineral and/or vitamin supplementation before natural mating can increase the reproductive performance in ewes. Combined selenium and vitamin E intra-muscular injections (Mudd and Mackie, 1973) or the use of slow-release rumen-blouses Se plus I, Co alone or combined Se/I plus Co (MAFF, 2000) before mating has been shown to increase multiple births. The results of the present study also showed that pregnancy and multiple births rates following the synchronization protocol in ewes receiving 2 boluses + eCG were 100% and 80%, respectively, which were higher than the above mentioned results. In the presence of rams in the flock, all ewes given the bolus and eCG injections became pregnant after the synchronization protocol or during their next estrus. This work suggests that mineral supplementation in ewes may increase pregnancy and multiple births rates. The investigation of slow-release ruminal boluses has also indicated that they may be of greater bioavailability (Hemingway *et al*., 2001; Kendall *et al*., 2001). Therefore, the potential exists for the enhanced bioavailability of ruminal boluses to boost reproductive efficiency, embryo numbers, embryo quality (MAFF, 2000; Mitchell *et al*., 2007) and multiple births (Hemingway *et al*., 2001).

In conclusion, boluses increased the status of copper, selenium and iodine both at mating time and days 90 to 100 of gestation. Following four weeks of mineral supplementation, which was the feeding protocol used in this experiment, multi-trace element ruminal boluses appeared not to significantly increase the number of different classes of ovarian follicles. Results showed that the group receiving 2 boluses + eCG showed higher multiple births rates in Iranian Afshari ewes during the breeding season.

Acknowledgement

Appreciation is expressed to Dr. R. Abdollahi-Arpanahi for his assistance in the various stages of this work.

References

Ammerman, CB; Henry, PR and Miles, RD (1998). Supplemental organically-bound mineral compounds in livestock nutrition. In: Garnsworthy, PC and Wiseman, J (Eds.), *Recent advances in animal nutrition*. UK, Nottingham University Press. PP: 67-91.

Barrett, DMW; Bartlewski, PM; Symington, A and Rawlings, NC (2004). Ultrasound and endocrine evaluation of the ovarian response to a single dose of 500 IU eCG following a 12-day treatment with progestagen-releasing intravaginal sponges in the breeding and non-breeding season in ewes. Theriogenology. 61: 311-327.

Bremner, I; Young, BW and Mills, CF (1976). Productive effect of zinc supplementation against copper toxicosis in sheep. Brit. J. Nutr., 36: 551-561.

Fisher, CEJ and MacPherson, A (1991). Effects of cobalt deficiency in the pregnant ewe on reproductive performance and lamb viability. Res. Vet. Sci., 50: 319-327.

Hackbart, KS; Ferreira, RM; Dietsche, AA; Socha, MT; Shaver, RD; Wiltbank, MC and Fricke, PM (2010). Effect of dietary organic zinc, manganese, copper, and cobalt supplementation on milk production, follicular growth, embryo quality, and tissue mineral concentrations in dairy cows. J. Anim. Sci., 88: 3856-3870.

Hartley, ED and Grant, AB (1961). A review of selenium responsive diseases in New Zealand livestock. Fed. Proc., 20: 679-688.

Hemingway, RG; Parkins, JJ and Ritchie, NS (2001). Enhanced reproductive performance of ewes given a sustained-release multi-trace element/vitamin ruminal bolus. Small Rumin. Res., 39: 25-30.

Hill, MK; Walker, SD and Taylor, AG (1969). Effects of marginal deficiencies of copper and selenium on growth and productivity in sheep. NZ. J. Agric. Res., 12: 261-270.

Kendall, NR; Jackson, DW; Makenzie, AA; Illingworth, DV; Gill, MI and Talfer, SB (2001). The effect of the zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter. Brit. Soc. Anim. Sci., 72: 163-169.

Koyuncu, M and Ozis Alticekic, S (2010). Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. Asian-Aust. J. Anim. Sci., 23: 308-311.

Lamb, CG; Brown, DR; Larson, JE; Dahlen, CR; DiLorenzo, N; Arthington, JD and DiCostanzo, A (2008). Effect of organic or inorganic trace mineral supplementation on follicular response, ovulation, and embryo production in superovulated Angus heifers. Anim. Reprod. Sci., 106: 221-231.

MAFF (2000). Ministry of Agriculture, Fisheries and Food. The effects of supplementing ewes with cobalt and iodine on ewe reproductive performance and lamb survival. MAFF Project Code: LS1508.

McDowell, LR (1992). *Mineral in animal and human nutrition*. 2nd Edn., London, Academic Press Ltd., PP: 186-201.

McGowan, AC (1983). The use of Lipiodol® for sub-clinical iodine deficiency in livestock - Brief Communication. Proc. N. Z. Soc. Anim. Prod., 43: 135-136.

Menchaca, A and Rubianes, E (2004). New treatments associated with timed artificial insemination in small ruminants. Reprod. Fertil. Dev., 16: 403-413.

Mitchell, LM; Robinson, JJ; Watt, RG; McEvoy, TG; Ashworth, CJ; Rooke, JA and Dwyer, CM (2007). Effects of cobalt/vitamin B12 status in ewes on ovum development and lamb viability at birth. Reprod. Fertil. Dev., 19: 553-562.

Mudd, AJ and Mackie, IL (1973). The influence of vitamin E and selenium on ewe prolificacy. Vet. Rec., 93: 197-199.

NRC (2007). National Research Council. Nutrient requirements of small ruminants: sheep-goats-cervids and new world camelids. 7th Rev. Edn., Washington D.C., USA, National Academy Press. PP: 221-229.

Parker, WJ and McCutcheon, SN (1989) Effects of iodine supplementation on the productivity of romney ewes in Waipara region of New Zealand. New Zealand J. Agric. Res., 32: 207-212.

Salehi, R; Kohram, H; Towhidi, A; Kermani Moakhar, H and Honarvar, M (2010). Follicular development and ovulation rate following different superovulatory treatments in Chall ewes. Small Rumin. Res., 93: 213-217.

SAS (1996). *User's Guide: Statistics*. Version 5th Edn., SAS Institute Inc., Cary, NC, USA.

Scales, GH (1974). Reproductive performance of Merino ewes dosed with selenium prior to mating. Proc. N. Z. Soc. Anim. Prod., 34: 103-113.

Shipley, CFB; Buckrell, BC; Mylne, MJA; Pollard, J and Hunton, JR (2007). Artificial insemination and embryo transfer in sheep. In: Threlfall, RSYR (Ed.), *Current therapy in large animal theriogenology*. (2nd Edn.), Saint Louis, W. B. Saunders. PP: 629-641.

Sirjani, MA; Kohram, H and Shahir, MH (2012). Effects of eCG injection combined with FSH and GnRH treatment on the lambing rate in synchronized Afshari ewes. Small Rumin. Res., 106: 59-63.

Story, C; Rasby, RJ; Brink, DR; Kinder, J and Moravek, T (1999). Trace mineral supplementation and ovarian and luteal function in pubertal heifers. Anim. Sci. Dep., Nebraska Beef Cattle Reports.

Underwood, EJ and Suttle, NF (1999). *The mineral nutrition of livestock*. 3rd Edn., Wallingford, Oxon, UK, CABI Publishing. PP: 285-332.

Upadhyay, SR; Singh, AK; Sharma, N; Kuma, PR; Hussain, K and Soodan, JS (2006). Impact of minerals upon reproduction in farm animals. Division of Clinical Veterinary Medicine and Jurisprudence, Faculty of Veterinary Science and Animal Husbandry. SKUAST-J, RS Pura, Jammu, 181-102 (J&K).