EFFECT OF PRE- AND POST-MATING VITAMINS AD3E TREATMENT ON REPRODUCTIVE PERFORMANCE OF AWASSI EWES T. A. Abdulkareem¹ S. M. Eidan¹ F. K. Al-Saidy² N. K. Al-Hassani² Prof. Prof. Researcher Researcher ¹ Depart. Anim. Prod., Coll. Agric. Engin. Sci., University of Baghdad. ² Direct. Agric. Ext. and Train., Min. Agric.

drtalalabdulkareem2013@gmail.com

ABSTRACT

This study was designed to investigate the influence of pre-estrus (PE) synchronization and post-mating (PM) vitamins AD₃E treatment on the reproductive performance of Awassi ewes raised at four Iraqi provinces. Seventy six Awassi ewes were randomly divided within each province into two groups. The first group (n=15, except for Karbala, n= 11) were im injected with five AD₃E injections (150 IU / dose), biweekly interval. The 1st injection was 1.5 months PM, while the 2nd and 3rd injections were at the time of progestagen-impregnated vaginal sponges insertion. The 4th injection was at the time of the vaginal sponge's removal, whereas the 5th injection was 14 days PM. The control group (n=5) was intramuscularly injected with 5 ml normal saline at similar periods. The AD₃E groups in Al-Najaf and Karbala provinces. Higher (P≤0.01) lambing rate and liter size were noticed in AD₃E groups than those of the control group for all provinces. In conclusion, pre-estrus synchronization and post-mating vitamin AD₃E treatment improved the reproductive performance of Awassi exes

Keywords: Vitamins, Mating, Reproduction, Ewes.

المستخلص

صممت هذه الدراسة بهدف بيان تاثير المعاملة بفيتامينات AD₃E قبل توحيد الشياع وبعد التسفيد في الاداء التناسلي للنعاج العواسي في اربعة محافظات عراقية. استخدمت 76 نعجة عواسية قسمت عشوائياً ضمن كل محافظة الى مجموعتين. عوملت AD₃E المعوموعة الاولى (15 نعجة / محافظة باستثناء كربلاء التي استخدمت فيها 11 نعجة) بخمس جرعات من فيتامينات AD₃E (محموعة الاولى (15 نعجة / محافظة باستثناء كربلاء التي استخدمت فيها 11 نعجة) بخمس جرعات من فيتامينات AD₃E (محموعة الاولى (15 نعجة / محافظة باستثناء كربلاء التي استخدمت فيها 11 نعجة) بخمس جرعات من فيتامينات AD₃E (محموعة الاولى (15 نعجة / محافظة باستثناء كربلاء التي استخدمت فيها 11 نعجة) بخمس جرعات من فيتامينات (100 وحدة دولية / جرعة) عن طريق العضلة وبواقع اسبوعين بين جرعة واخرى. حقنت الجرعة الاولى قبل 1.5 شهر من موعد تسفيد النعاج، تلتها الجرعة الثانية والثالثة مع وقت دفع الاسفنجات المهبلية المشبعة بهرمون البروجستيرون الصناعي (100 ملغم / اسفنجة). حقنت الجرعة الثانية والثالثة مع وقت محب الاسفنجات المهبلية، في الوقت الذي حقنت فيه الجرعة الخامسة بعد مرور 14 يوم من موعد الثانية والثالثة مع وقت محب الاسفنجات المهبلية، في الوقت الذي حقنت فيه الجرعة الخامسة بعد مرور 14 يوم من موعد التسفيد. اما المجموعة الثانية ضمن كل محافظة (5 نعاج/ محافظة) فقد حقنت بالمحلول الفسيولوجي (5 مللتر) عند الاوقات نفسها لدى المجموعة الاولى وعدت كمجموعة سيطرة. اظهرت مجموعة AD₃ الفسيولوجي (5 مللتر) عند الاوقات نفسها لدى المجموعة الاولى وعدت كمجموعة اسيطرة. اظهرت مجموعة الخامسة الفسيولوجي (5 مللتر) عند الاوقات نفسها لدى المجموعة الاولى وعدت كمجموعة اسيطرة. اظهرت مجموعة AD اعلى الفسيولوجي (5 مللتر) عند الاوقات نفسها لدى المجموعة الاولى وعدت كمجموعة السيطرة. الفيرة الذي تميزت فيه العلى المعاي موجموعة الاولى وعدت كمجموعة المورة الفي مجموعة AD العلى المحلول (100 ≥ P) نسبتي خاصية مقارنة محاميع السيطرة لدى محافظتي كربلاء والنجف، في الوقت الذي تميزت فيه موموعة AD موجموعة السيطرة الذى محموعة السيطرة لدى محافظة موري AD موجموعة الدي محموعة المورة الدى محموعة الموموعة الال مولي ولال وعال مومو مولية الدى المحموعة الولى وعدت كمجموعة المومة الفي مجموعة الذي تموموا الذي محموعة الدي محمومة الذي محموعة الدي مولي ولي موليا ولمو

الكلمات المفتاحية: الفيتامينات، التسفيد، التناسل، النعاج.

Received:27/6/2021, Accepted:12/9/2021

INTRODUCTION

The sheep sector in Iraq lacks its ability to compete in global markets through the low productivity of milk and meat. The lack of locally-produced feed, overgrazing, ignorance of most owners with the importance of the flushing periods for the sheep reproductive life, and inappropriate managmental and nutritional practices used (2, 4, 7, 8, 9). In Iraq, the sheep mating season generally lasts from May to October. At this time, sheep are grazing cereal stubble usually and supplemented with straw and grain only that deficient in vitamin A (6, 7). This vitamin exists in green roughages and, is stored in the liver for 3-4 months (17), and this level may be possibly reduced toward the end of the mating season when green roughages are unavailable Consequently, (3). the reproductive performance of Iraqi sheep could be affected by the lack of vitamin A in the diet (6, 14). Estrus synchronization is one of the most efficient techniques in improving the reproductive efficiency and productivity of sheep by reducing the effort exerted in providing reproductive care to ewes during the breeding season and producing lambs of consistent ages and weights prepared for marketing (4, 5). Using of vitamins AD₃E, preand post-estrus synchronization as well as post-mating will increase the viability of ovarian follicles and enhance ovulation and conception rates consequently (8). This was not addressed in previous studies, which were limited to using it pre-mating exclusively in Iraq and Arab countries (1, 6). Moreover, conducted of this study on the extension farmwide and owners has not previously been investigated. Therefore, this study was undertaken to explore the effect of pre-estrus synchronization (PE) and post-mating (PM) vitamins AD₃E treatment on the reproductive performance of Awassi ewes at the extension demonstrating farms.

MATERIALS AND METHODS Experimental animals and treatments

This study was conducted by sheep owners at four Iraqi provinces, namely Al-Najaf (Al-Haydaria Farm), Wasit (Al-Ezza Farm), Muthanna (Al-Majd Farm), and Karbala (Al-Sahrawia Farm). Seventy-six Awassi ewes were divided within each province into two groups. The first group (n=15, except for Karbala, n=11) were im injected with AD₃E (150 IU / injection), biweekly interval between each injection. The 1st injection was 1.5 months PM, while the 2^{nd} and 3^{rd} injections were at the time of progestagen-impregnated vaginal sponges (60 mg medroxyprogesterone acetate) insertion. The 4th injection was at the time of the vaginal sponge's removal, whereas the 5th injection was 14 days PM. The control group within each province (n=5) was im injected with 5 ml normal saline at similar dates mentioned in AD₃E injected groups. Ewes were fed on a concentrate diet containing 14 % crude protein and 2500 Kcal per kg concentrate consisting of barley grains; 44%, wheat bran; 20%, maize; 20%, soybean meal; 13% as well as salts, CaCl₃, vitamins, and minerals; 3% with some green roughage according to the season (Alfalfa and barely grass) at 2% of live body weight (17). This pattern of feeding continued for 60 days (45 days pre-mating (PR) and 15 days PM), while the animals were left to graze until lambing for both treatment and control groups. Fertility (%), litter size, and the rates of conception, lambing, and twinning together with barrenness were calculated according to Al-Saigh & Alkass (10). Fecundity rate, extra lambs, and increasing fecundity percentage were estimated according to Palacin et al (18).

Statistical analyses

Data were analyzed using a general linear model (GLM) procedure (20) to investigate the effect of vitamin AD_3E treatment on the reproductive performance of Awassi ewes. The statistical model for the analysis of variance was:

 $Y_{ij} = \mu + T_i + e_{ij}$

Where:

 Y_{ij} = dependent variables (reproductive performance characteristics, fecundity rate, extra lambs and increasing in fecundity percentage).

 $\boldsymbol{\mu} = Overall \ mean$

 T_i = Effect of treatment (AD3E and control groups).

 $e_{ij} = Error term$

Differences among means were computed using Duncan multiple range test (13). Chisquare was used to compare the significant differences among the percentages of reproductive performance characteristics (22).

RESULTS AND DISCUSSION

Estrus response rate

The estrus response rate did not differ significantly among treated and control groups pertaining to the involved provinces. Moreover, this percentage did not differ among provinces within each treatment, being 100 % for treated and control groups (Table 1). The non-significance between AD_3E treated and control groups may be due to the fact that both groups are responding to the Table 1 Effect of vitaming AD E treatment or

synchronization estrus technique using progestagen-impregnated sponges with good efficiency. On the other hand, the good feeding of ewes' pre-and during mating contributed to exhibiting good estrus percentages in both groups. The PR flushing stimulates the secretion of the gonadotropins from the pituitary gland (11), progesterone from the corpus luteum (11, 16) and estradiol (19), as well as its role in growth hormone and insulin secretion (12), thus stimulating the appearance of estrus and improving the ovulation rate consequently (21).

 Table 1. Effect of vitamins AD₃E treatment on estrus response rate of Awassi ewes in different Iraqi provinces.

Province	AD ₃ E group	Control group	Chi-square (χ2)
Al-Najaf	100	100	0.00 (NS)
Wassit	100	100	0.00 (NS)
Muthanna	100	100	0.00 (NS)
Karbala	100	100	0.00 (NS)
Total	100	100	0.00 (NS)
Chi-square (χ2)	0.00 (NS)	0.00 (NS)	

NS= Non-significant.

Fertility rate: The fertility rate was greater ($P \le 0.01$) for the AD₃E-treated group as compared with the control group for Al-Najaf (100 vs. 60 %) and Karbala (64 vs. 40%) provinces as well as for the total number (87.5 vs. 70%). This percentage between treated and control groups did not differ in Wassit and Muthanna provinces (Table 2). These results were inconsistent with those reported by

Abdelrahman & Al-Karablieh (1) in Jordanian Awassi ewes, where the fertility rate increased significantly ($P \le 0.01$) from 74.1 % for the control group to 82.1% for the ewes treated with AD₃E, week 2-3 PR. The reason behind this inconsistency may return to the number of animals as well as management practices in each study.

Table 2. Effect of vitamins AD ₃ E treatment on fertility rate of Awassi ewes in different Iraqi	
provinces.	

Provinces	AD ₃ E group	Control group	Chi-square (χ2)
Al-Najaf	<u> </u>	<u>60</u>	9.46 ($P \le 0.01$)
Wassit	80	80	0.00 (NS)
Muthanna	100	100	0.00 (NS)
Karbala	64	40	8.17 (P≤ 0.01)
Total	87.5	70	6.94 (P≤0.01)
Chi-square (χ2)	9.02 (P≤0.01)	11.85 (P≤ 0.01)	

NS= Non-significant.

Conception rate

A similar trend was noticed for the conception rate as for the fertility rate. The vitamins AD3E group had a greater (P \leq 0.01) rate as compared to the control group in Al-Najaf and Karbala provinces, while the differences among these groups lacked significance in Wassit and Muthanna provinces (Table 3). These results also clearly indicate the efficiency of sheep owners in Al-Najaf and Karbala provinces in applying vitamins AD₃E and estrus synchronization techniques in an efficient manner compared to sheep owners in Wassit and Muthanna provinces. This prompted us to encourage those owners by providing support to them and conducting field visits and scientific seminars in order to improve the productivity of their animals for the better. The higher total fertility and conception rates of the AD₃E-treated group by 25% compared to the control group indicates the importance of using five doses of vitamins AD₃E (150 IU / dose) PR accompanied with estrus synchronization to improve the fertility and conception rates of Awassi sheep

Table 3. Effect of vitamins AD ₃ E treatment on conception rate of Awassi ewes in different
Iraqi provinces.

Provinces	AD ₃ E group	Control group	Chi-square (χ2)
Al-Najaf	100	60	9.46 (P≤ 0.01)
Wassit	80	80	0.00 (NS)
Muthanna	100	100	0.00 (NS)
Karbala	64	40	8.17 (P≤ 0.01)
Total	87.5	70	6.94 (P≤0.01)
Chi-square (χ2)	9.02 (P≤0.01)	11.85 (P≤ 0.01)	

NS= Non-significant

Lambing rate: A greater (P≤0.01) lambing rate was observed for the AD₃E-treated groups in comparison with the control groups in all provinces (Table 4). Similarly, higher ($P \le 0.01$) lambing rates were found in Al-Najaf (180%) and Muthanna (100%) provinces while, the lesser rate in Karbala (64 and 40 %; Table 4). These results were inconsistent with those observed by Al-Haboby et al (6), where a greater lambing rate was noticed for Awassi ewes treated with AD₃E than those of the control group (64.7 vs. 56.7 %). The current results are also in line with what Abdelrahman & Al-Karablieh (1) indicated; being higher lambing rate of the Jordanian ewes treated with AD₃E than those of the control ewes

(86.7 vs. 77.9%). The obvious increase in the total lambing rate of the treated group compared to the control group (+91%) clearly reflects the increase in the number of lambs born as a result of using AD₃E treatment preand post-estrus synchronization, which is positively reflected in the increase in the number of marketing lambs per season by the owner and his economic return improved accordingly. Increasing the lambing rate of the AD₃E-treated group reflects a large extent, an increase in ovulation rate decreasing in early embryonic death, and consequently, the success of the development and growth of the embryos further (15).

Table 4. Effect of vitamins AD3E treatment on lambing rate of Awassi ewes in different Iraqi
provinces.

	piotin		
Provinces	AD ₃ E group	Control group	Chi-square (χ2)
Al-Najaf	180	60	32.74 (P≤0.01)
Wassit	113.3	80	9.42 (P≤ 0.01)
Muthanna	160	100	17.51 (P≤ 0.01)
Karbala	64	40	8.17 (P≤ 0.01)
Total	133.93	70	17.04 (P≤0.01)
Chi-square (χ2)	36.08 (P≤0.01)	12.69 (P≤0.01)	

Twinning rate

The AD₃E-treated group exhibited a greater (P<0.01) twinning rate for Al-Najaf (80%), Wassit (41.66%), Muthanna (60%) as well as a total percentage (53.06%) compared to 0.0% for the control groups of similarly mentioned provinces (Table Non-significant 5). differences were observed between treated and control groups for Karbala province (Table 5). On the other hand, highly significant ($P \le 0.01$) differences were noticed among the provinces within each group, being highest in Al-Najaf (80%) and lowest in Karbala (0.0%) provinces (Table 5). Similar results have been obtained by Al-Haboby et al (6) who found a greater twinning rate for Awassi ewes treated with AD_3E as compared to the control group (3.8)

vs. 2.5%). These results were also similar to what Abdelrahman & Al-Karablieh (1) found in terms of the superiority of Jordanian Awassi ewes treated with vitamins AD₃E over the ewes of the control group in twinning rate (5.3 vs. 4.3 %). This obvious increase in the total twinning rate (53.06%) reflects an increase in the number of lambs born compared to the control group and thus, increases the economic return of the sheep owners. In line with the aforementioned results, the provinces of Al-Najaf and Muthanna achieved the highest twinning rate (80%) compared to those of Wasit and Karbala, which indicates the excellence of the owners of these two provinces and their adoption of modern techniques in raising their sheep flocks.

	provin	ices.	
Province	AD ₃ E group	Control group	Chi-square (χ2)
Al-Najaf	80	0.00	18.63 (P≤0.01)
Wassit	41.66	0.00	9.75 (P≤ 0.01)
Muthanna	60	0.00	17.51 (P≤0.01)
Karbala	0.0	0.00	0.00 NS
Total	53.06	0.00	13.58 (P≤0.01)
Chi-square (χ2)	16.72 (P≤0.01)	0.00 NS	

Table 5. Effect of vitamins AD ₃ E treatment on twinning rate of Awassi ewes in different Iraqi
provinces.

NS= Non-significant. Litter size

Greater (P≤0.01) litter size was noticed for vitamins AD₃E-treated groups in comparison with the control group in all involved provinces, as well as the overall mean (1.34 vs. 0.7; Table 6). On the other hand, nonsignificant differences were observed in litter size between Al-Najaf and Muthanna provinces within the AD₃E-treated group which recorded the highest value (1.8) compared to Wassit and Karbala provinces which recorded the lesser values, namely 1.42 and 1.0 respectively (Table 6). The great role that vitamin A plays in the development of ovarian follicles, ovarian activity, and the

improvement of the ovulation rate (14) was clearly reflected in the increase in litter size of ewes treated with vitamins AD_3E through increasing the rate of pregnancy and the successful development of embryos (15). Moreover, the role of vitamin E in improving the immune response, reducing the damage of oxidative stress, and their relation with enhancing the ovarian follicles (14) improved the litter size of treated ewes. Increasing litter size among treated ewes will be positively reflected increasing in marketing lambs postweaning, thus increasing the economic income of sheep owners in these provinces.

Table 6. Effect of vitamins AD ₃ E treatment on litter size of Awassi ewes in different Iraqi
provinces.

	P10/1		
Province	AD ₃ E group	Control group	Significance level
Al-Najaf	1.8 a	0.6 c	P≤0.01
Wassit	1.42 b	0.8 ab	P≤ 0.01
Muthanna	1.8 a	1.0 a	P ≤ 0.01
Karbala	1.0 c	0.4 d	P≤ 0.01
Total	1.34 b	0.7 bc	P≤ 0.01
Significance level	(P ≤ 0.01)	(P≤0.01)	

Fecundity rate

Treatment with vitamin AD₃E had а significant ($P \le 0.01$) increase in fecundity rate compared to the control group in Al-Najaf, Karbala provinces. Wassit. and The differences between the two groups in Muthanna province lacked significance; however, numeral increasing (60%) of the fecundity rate in treated vs. the control groups (Table 7). On the other hand, a greater (P \leq 0.01) fecundity rate was observed among the provinces within the treated group, being higher in Al-Najaf (1.80) and Muthanna (1.60) provinces than Wassit (1.133) and Karbala (0.636) provinces (Table 7).These results confirmed those obtained for litter size, being greater in treating ewes of Al-Najaf and Muthanna than those of Wassit and Karbala provinces.

Table 7. Effect of vitamin AD ₃ E treatment on fecundity rate of Awassi ewes in different Iraqi
nrovinces

provinces.			
Province	AD ₃ E group	Control group	Significance level
Al-Najaf	1.80 a	0.6 bc	(P≤0.01)
Wassit	1.133c	0.8 ab	(P ≤ 0.01)
Muthanna	1.60 ab	1.0 a	NS
Karbala	0.636 d	0.4 c	(P≤0.01)
Total	1.339 b	0.7 a	(P≤0.01)
Significance level	(P≤0.01)	(P≤0.01)	

NS= Non-significant.

Extra lambs and increasing in fecundity percentage: Confirming the fecundity rate results (Table 7), ewes of Al-Najaf province exhibited the highest ($P \le 0.01$) increasing fecundity percentage as a result of vitamin AD₃E treatment reached 200%, followed by ewes of Muthanna (60%), Karbala (59%) and Wassit (41.6%) provinces respectively. The overall mean of this percentage for AD₃E treatment is 91.3% (Table 8). Moreover, the ewes for Al-Najaf province achieved a greater ($P \le 0.01$) number of extra lambs among provinces reached 1.2, followed by Muthanna (0.60) and Wassit (0.333) provinces. The ewes of Karbala province recorded the lowest (P≤0.01) number of extra lambs was 0.236 (Table 8). The number of extra lambs is one of the most important traits in assessing the economic income achieved by sheep owners in the world (18). The greater lambing and twinning rates in Al-Najaf and Muthanna provinces were clearly reflected in the increase of extra lambs born to them. This will consequently increase the number of marketing ram lambs as well as improve the chance of regenerating the flock for extra ewe lambs born (10).

Table 8. Effect of vitamins AD ₃ E treatment on extra lambs and increasing in fecundity
percentage of Awassi ewes in different Iragi provinces

Province	Extra lambs	Increasing in fecundity percentage	
Al-Najaf	1.2 a	200 a	
Wassit	0.333 bc	41.6 c	
Muthanna	0.60 b	60 c	
Karbala	0.236 c	59 c	
Total	0.639 b	91.3 b	
Significance level	(P≤0.01)	(P≤0.01)	

REFERENCES

1. Abdelrahman, M. M. and E. Al-Karablieh. 2002. Effect of vitamin AD_3E injection on reproductive performance and net cash revenue of Awassi sheep raised under semiextensive system. J. King Saud Univ. Sci., 14(1), 15-22.

2. Abdulkareem, T.A. 2002. Reproductive performance of Awassi ewe lambs following treatment with equine chorionic gonadotropin. Iraqi Journal of Agricultural Science, 33 (2), 263-266.

3. Abdulkareem, T.A., A. H. Al-Haboby, S. M. Al-Mjamei and A. A. Hobi. 2005. Sperm abnormalities associated with vitamin A deficiency in rams. Small Rumin. Res., 57 (1), 67–71

4. Abdulkareem, T.A., S. M. Eidan, L. A. Al-Maliki, F. K. Al-Saidy and M. R. Mahdi. 2014. Reproductive performance of Iraqi Awassi ewes owned by producers and extension farms in response to flushing and estrous synchronization regimes. Iraqi Journal of Agricultural Science, 45(3), 328-334.

5. Abdulkareem. T. A., S. J. Muhammad and A. N. Yousif. 2021. Effect of kisspeptin-10 as an alternative to eCG in estrus synchronization protocol on improving the reproductive performance of Karadi ewes. Iraqi Journal of Agricultural Science, 52 (3), 535-546. https://doi.org/10.36103/ijas.v52i3.1340

6. Al-Haboby, A.H. T. A. Abdulkareem and G. K. Khatab. 1997. Effect of vitamin A on the reproductive performance of Awassi sheep. Regional Symposium of Integrated Crop – Livestock System in the Dry Area of West Asia and North Africa, 6-8 Nov., Amman– Jordan, pp. 300.

7. Al-Haboby, A.H., A. D. Salman and T. A. Abdulkareem. 1999. Influence of protein supplementation on reproductive traits of Awassi sheep grazing cereal stubble. Small Rumin. Res., v.34, n.1, p.33-40,

8. Alkass, J.E. T. A. Abdulkareem and S. M. Al-Mjamei. 2004. Reproductive performance of Iraqi Awassi ewes in response to treatment with equine chorionic gonadotropin. J. Agric. Invest., 2, 74–77.

9. Alkass, J.E., A. J. Pedawy and T. A. Abdulkareem 2013. Effect of different dietary protein levels and age on testicular development and ultrastructures in Karadi ram lambs. International Journal of Agricultural and Food Research (Canada), 2 (4), 14-24

10. Al- Saigh, M. N. and J. E. Alkass. 1992. Sheep and Goat Production. Dar Al- Hikma Press. 11. Boland, M. P., P. Longergan, and D. O'Callaghan. 2001. The effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology, 55 (6), 1323-1340

12. Downing, J., J. Joss, R. J. Scaramuzzi. 1995. Ovulation rate and the concentrations of gonadotrophins and metabolic hormones in ewes infused with glucose during the late luteal phase of the oestrous cycle. J. Endocrinol., 146 (3), 403-410,

13. Duncan, D. 1955. Multiple range and multiple F-tests. Biometrics, 11(1), 1–24

14. Liu, S., D. Masters and M. Ferguson, and A. Thompson. 2014. Vitamin E status and reproduction in sheep: potential implications for Australian sheep production. Anim. Prod. Sci., 54 (6), 694-714.

15. Martin, G. B., J. T. B. Milton, R. H. Davidson, G. E. Banchero Hunzicker, D. R. Lindsay and D. Blache. 2004. Natural methods for increasing reproductive efficiency in small ruminants. Anim. Reprod. Sci., 82–83, 231–245

16. McEvoy, T. G., J. Robinson, R. P. Aitken, P. A. Findlay, R. Palmer and I. S. Robertson. 1995. Dietary induced suppression of preovulatory progesterone concentration in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. J. Anim. Reprod. Sci., 39(2), 89-107. 17. NRC. 1985. Nutrient Requirement of sheep. 6th revised ed. National Research Council, Washington, D.C. pp. 22-23.

18. Palacin, I., J. A. Abecia, F. Forcada, A. Casao, J. A. Cebrian, T. Muino, C. Palacios and J. M. Pontes. 2008. Effect of exogenous melatonin treatment on out-of season ram fertility. Ital. J. Anim. Sci., 7(2), 199-206.

19. Payan, E., J. F. Smith, B. C. Copeand and L. T. McGowan. 1991. Studies on the role of liver cytochrome and estradiol metabolism in the effect of nutrition and Phenobarbital on ovulation rate in the ewe. Reprod. Fertil. Dev., 3 (6), 725-736.

20. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

21. Somchita, A. B. K. Campbell, M. Khalid, N. R. Kendall and R. J. Scaramuzzi. 2007. The effect of short-term nutritional supplementation of ewes with lupin grain (*Lupinus luteus*), during the luteal phase of the estrous cycle on the number of ovarian follicles and the concentrations of hormones and glucose in plasma and follicular fluid. Theriogenology, 68 (7), 1037-1046.

22. Steel, R.G.D and J. H. Torrie. 1990. Principles and Procedures of Statistics. A biometrical approach. 3rd edn. Tokyo: McGraw-Hill, Kogakusha Ltd.