

EFFECT OF SELENIUM SUPPLEMENTATION ON TWINNING INDUCTION IN BARKI EWES

HOWIDA M.A. ABD-EL-RAHMAN¹ and MAHA A. IBRAHIM²

¹ Field Investigation Department, Animal Reproduction, Research Institute (ARRI), Giza, Egypt

² Biology of Reproduction Department, Animal Reproduction, Research Institute (ARRI), Giza, Egypt

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ABSTRACT

This study aimed to evaluate selenium supplementation effect on induction of twinning of Barki ewes by using two different doses of PMSG in estrus synchronization program during the breeding season. For the experiment, 28 ewes were randomly divided into four groups, 7 ewes in each group, all ewes were treated with intra-vaginal sponges, containing 60 mg medroxy progesterone acetate for 14 days, immediately after sponge withdrawal, PMSG hormone at a doses of 250 IU in groups I, II and 750 IU in groups III and IV were administered intramuscularly. Selenium (Se) levels were 0.05 mg/kg diet DM (without supplementation) for groups I and III and 0.2 mg /kg diet DM for groups II and IV (Se supplementation). The pregnancy and barren rates for groups I, II, III and IV were (57.14%, 100%, 71.43% and 85.71 %) and (42.86%, 0.0%, 28.57% and 14.29%), respectively ($P < 0.01$). At the same time, the twinning rate was (0%, 71.43%, 42.86% and 71.43%), and the lambing rate was (57.14%, 100%, 71.43% and 85.71 %) for the four groups respectively ($P < 0.01$). Meanwhile, the fecundity rate for the same groups was (57.14%, 171.43%, 114.28% and 157.14%), respectively. Decreasing PMSG dose with Se supplementation appear to be a most appropriate program for induction of twinning and increasing of fertility and fecundity rates.

Key words: selenium, twinning rate, fertility, fecundity, ewes.

INTRODUCTION

Methods to improve reproduction in sheep often aim to increase the proportion of ewes having multiple ovulations, and thereby increase lambing percentage (Wilkins, 1997). Increasing rate of fecundity in sheep offers the best opportunity to increase the efficiency of lamb meat production (Turk *et al.*, 2008). Administration of gonadotropins such as human chorionic gonadotropin (hCG) (Evans, 2003), pregnant mare serum gonadotropin (PMSG) (Lamrani *et al.*, 2008), follicle stimulating hormones (FSH) and mixed gonadotropin preparations (Kinghts *et al.*, 2003) after stopping progestogens treatment, causes increasing of ovulation and lambing rate. Between all endocrine approaches to increase twinning rate, administration of PMSG is more usual than others. Moreover, it was reported that PMSG can increase pregnancy and twinning rates in breeds characterized by low litter size (Boscos *et al.*, 2002). PMSG has a high affinity with both follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptors in the

ovaries and has long-lasting LH- and FSH-like effects on the follicle. It enhances the entry rate of small and medium follicles into larger follicles and it may prevent the occurrence of natural follicular atresia (Mandiki *et al.*, 2000). However, there are many factors influence the efficiency of PMSG, including the dose and administration time (Timurkan and Yildiz, 2005). It has been found that using of low dose of PMSG is less effective and did not increase fertility or prolificacy (Romano *et al.*, 1996 and Akoz *et al.*, 2006), and the using of high dose of PMSG induced multiple ovulations and improved the fertility and proliferation (Koyuncu *et al.*, 2001 and Timurkan and Yildiz, 2005). Meanwhile, as hormonal treatment is still expensive so that the use of high dose tends to elevate the costs for subsistence farmer, therefore the efficiency is difficult to achieve, as the major problem that faces the farmer in sheep reproduction management, is the looking for reproductive techniques, which could help the farmer, and decreases the cost of production. So that this problem motivates the researchers to find an alternative by using a lesser dose of gonadotropin, that implies a reduction in cost, without any reduction in fertility and proliferation.

Selenium is a micro mineral that is a part of the enzyme glutathione peroxidase (GSH-Px), which is

Corresponding author: Dr. MAHA A. IBRAHIM

E-mail address: maha_doctor2013@yahoo.com

Present address: Biology of Reproduction Department, Animal Reproduction, Research Institute (ARRI), Giza, Egypt

an important antioxidant that inactivates free radicals and that protects cells from the damage caused by lipid peroxidation and since protects the oocyte membrane against oxidative damage (Luberda, 2005; Livingston *et al.*, 2009). Se deficiency plays a role in numerous economically important livestock problems that include impaired fertility, abortion, retained placenta and neonatal weakness (McDowell *et al.*, 1996). Single injection of Se before mating and lambing significantly affects oestrous, fertility, and lamb daily weight gain (Gabryszuk and Klewec, 2002). Also, Malecki *et al.* (2002) stated that Se supplementation to ewes before mating improved fertility, prolificacy and the number of twins when compared with the control.

The objective of this study is to determine the impact of Se supplementation (0.05 or 0.2 mg /kg diet DM) on reproductive performance (pregnancy rate; twinning rate; lambing rate as well as fecundity rate) of adult ewes at a synchronized estrus in relation to two different doses of PMSG (high; 750 IU & low; 250 IU). And study the capability of combination between low dose of PMSG (250 IU) and Se

supplementation in terms of increasing the pregnancy and twinning rates in Barki ewes with a minimum cost.

MATERIALS AND METHODS

Animals and study site:

Twenty-eight multiparous clinically healthy Barki ewes weighing (50-60 kg) reared in a private farm were used in this study; the ewes were run with vasectomized rams and ascertained to be cyclic before the onset of the experiment.

Animal housing and feeding:

Each group was kept in a separate, well-ventilated shed, under condition of natural day light and temperature. All ewes were proved to be clinically healthy and free from external and internal parasites. All groups fed a standard total mixed ration according to NRC (1985). Feed composition of the used total mixed ration as shown in table (1). The ration was analyzed for Dry matter, Crude protein, Ash, Ether extract, NDF and ADF according to A.O.A.C. (1990).

Table 1: Feed composition and proximate analysis of the used diets (total mixed rations) for ewes under experiment:

Ingredient	Diet used during the first 4 months of pregnancy (% As- fed basis)		Diet used during the last 4 weeks of pregnancy (% As- fed basis)	
	Without Se supplementation	With Se supplementation	Without Se supplementation	With Se supplementation
Yellow Corn	4.00	4.00	-	-
Soybean meal (44%)	3.00	3.00	1.00	1.00
Wheat bran	4.5	4.5	16.00	16.00
Corn silage	82.1	82.1	61.00	61.00
Barseem straw	4.00	4.00	21	21
Mineral & Vitamin mixture:				
Mineral & Vitamin mixture(1)*	0.1	-	0.1	-
Mineral & Vitamin mixture with Se (2) **	-	0.1	-	0.1
Proximate analysis (on DM basis):				
Dry matter (%)	52.62	52.62	40.67	40.67
Crude protein (%)	14.03	14.03	10.60	10.60
Metabolizable energy (Mcal / kg)	2.50	2.50	2.10	2.10
Selenium (ppm)	0.05	0.20	0.05	0.20
Dry matter intake (kg / head/day):				
Dry matter (kg)	2.5 kg		1.8 kg	

* Each 1 Kg of mineral & Vitamin mixture (1) containing 2.5 g Zinc, 20.0g manganese, 2.00 g copper, 50.0 g iron, 0.25 g Iodine, 0.05 g Cobalt, 4000000 IU Vitamin A, 1000000 IU Vitamin D, and 15.0 g vitamin E.

** Each 1.0 kg of mineral and Vitamin (2) containing nutrient concentration as (1) in addition to 0.075 g Selenium.

Estrous synchronization and PMSG treatment:

All ewes were received intravaginal sponges impregnated with 60 mg medroxy progesterone acetate (Veramex®, Upjohn Company) for 14 days. At the day of sponges' withdrawal, ewes were randomly divided into four groups (GP) and an intramuscular administration of pregnant mare serum gonadotrophin, PMSG (Folligon, Intervet) as follow:

- The first group, (GPI; n = 7) ewes were injected with 250 IU PMSG / head without selenium supplementation.
- The second group, (GPII; n = 7) ewes were injected with 250 IU PMSG / head with selenium supplementation (selenium level 0.2 mg/kg diet DM).
- The third group, (GPIII; n = 7) ewes were injected with 750 IU PMSG / head without selenium supplementation.
- The fourth group, (GPIV; n = 7) ewes were injected with 750 IU PMSG / head with selenium supplementation (selenium level 0.2 mg/kg diet DM).

Estrus detection and mating:

Two fertile rams were introduced to all ewes in the four groups twice daily, just after sponge withdrawal, and left with them for natural mating for 2 hours each time.

Reproductive performance:

One month after the insemination, conception rates of animals of the four groups were checked by ultrasonography, using a real time B-mode (Vestron, Kontron France) equipped with 6.5 MHZ probe. Lambing was determined by daily observation and identification of ewes in small lambing plots. The numbers of lambs born per ewe were recorded during lambing.

Data analysis:

Data were subjected to statistical analysis by Microstate copy right (c) 1984; Eco soft, Inc. employing a completely randomized design according to Snedecor and Cochran (1982).

Measured traits throughout the experimental period:

Fertility was monitored in terms of pregnancy rate, twinning rate, lambing rate, as well as fecundity rate

(Hartley. 1963, Zeleke *et al.*, 2005, Kridli *et al.*, 2006 Ozyurtlz *et al.*, 2011).

Pregnancy rate = number of pregnant ewes / number of mated ewes x 100.

Barren rate = number of non-pregnant ewes / number of mated ewes x 100.

Twinning rate = number of pregnant ewes bearing twins/number of mated ewes x 100.

Lambing rate = number of lambing ewes / number of mated ewes x 100.

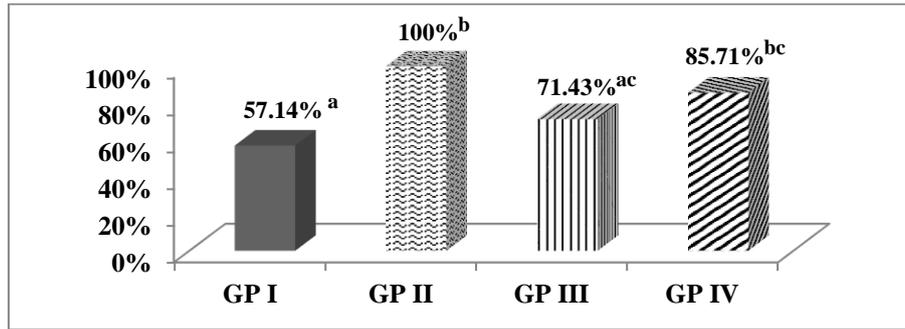
Fecundity rate = number of lambs born (alive) / number of ewes mated x 100.

RESULTS

No intravaginal sponges were lost during the trial and none of the ewes showed oestrous while the intravaginal sponges were in place.

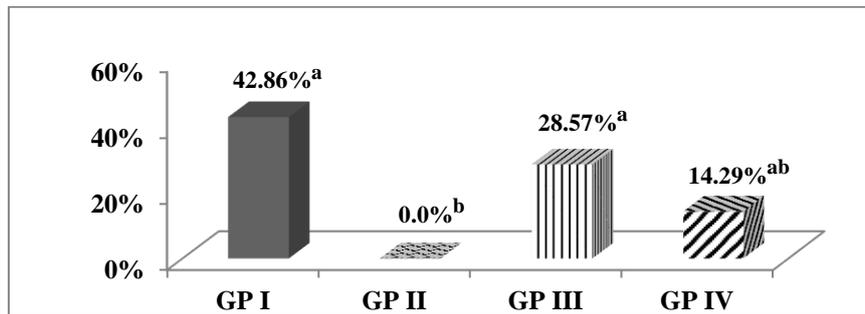
The effects of selenium supplementation and PMSG dose on reproductive performance are shown in Figures (1-5).

The results indicated that the pregnancy rate was significantly higher ($p < 0.05$) in group II than in group I (100% vs 57.14%) and tend to be significant ($p < 0.06$) than in group III (100% vs 71.43%), meanwhile it was non-significantly higher than in group IV (100% vs 85.71%). The twinning rate was significantly higher ($p < 0.05$) in group II than in group I (71.43% vs 0.0%), meanwhile it was non-significantly higher than in group III (71.43% vs 42.86%) and group IV (71.43% vs 71.43%). The barren rate was significantly lower ($p < 0.05$) in group II than in groups I (0.0% vs 42.86%) and III (0.0% vs 28.57%), meanwhile the decrease was non-significant than group IV (0.0% vs 14.29%). The fecundity rate was significantly higher ($p < 0.05$) in group II than in groups I (171.43% vs 100%) and III (171.43% vs 114.28%), meanwhile it was non-significantly higher than group IV (171.43% vs 157.14%). The lambing rate was significantly higher ($p < 0.05$) in group II than group I (100% vs 57.14%) and tend to be significant ($p < 0.06$) than in group III (100% vs 71.43%), meanwhile it was non-significantly higher than group IV (100% vs 85.71%). Also our results showed that pregnancy and lambing rates were tend to be significant ($p < 0.1$) in group IV than in group I (85.71% vs 57.14%).



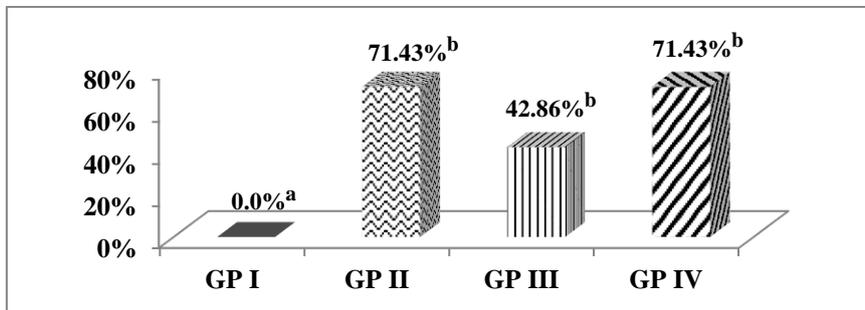
GP: group, significance at (P< 0.05)

Figure 1: Pregnancy rate of ewes (n=7) as affected by Se and PMSG (GP I) 250 IU PMSG without Se, (GP II) 250 IU PMSG with Se, (GP III) 750 IU PMSG without Se and (GP IV) 750 IU PMSG with Se.



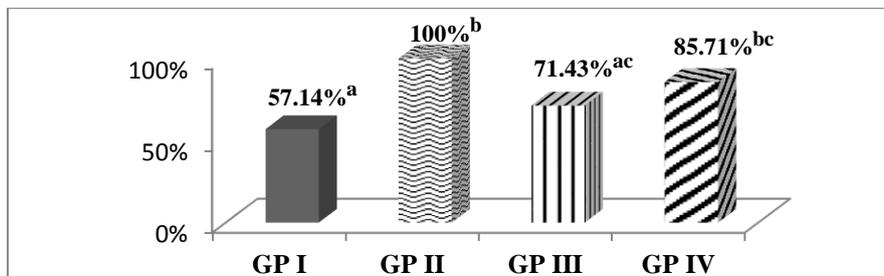
GP: group, significance at (P< 0.05)

Figure 2: Barren rate of ewes (n=7) as affected by Se and PMSG (GP I) 250 IU PMSG without Se, (GP II) 250 IU PMSG with Se, (GP III) 750 IU PMSG without Se and (GP IV) 750 IU PMSG with Se.



GP: group, significance at (P< 0.05)

Figure 3: Twinning rate of ewes (n=7) as affected by Se and PMSG (GP I) 250 IU PMSG without Se, (GP II) 250 IU PMSG with Se, (GP III) 750 IU PMSG without Se and (GP IV) 750 IU PMSG with Se.



GP: group, significance at (P< 0.05)

Figure 4: lambing rate of ewes (n=7) as affected by Se and PMSG, (GP I) 250 IU PMSG without Se, (GP II) 250 IU PMSG with Se, (GP III) 750 IU PMSG without Se and (GP IV) 750 IU PMSG with Se.

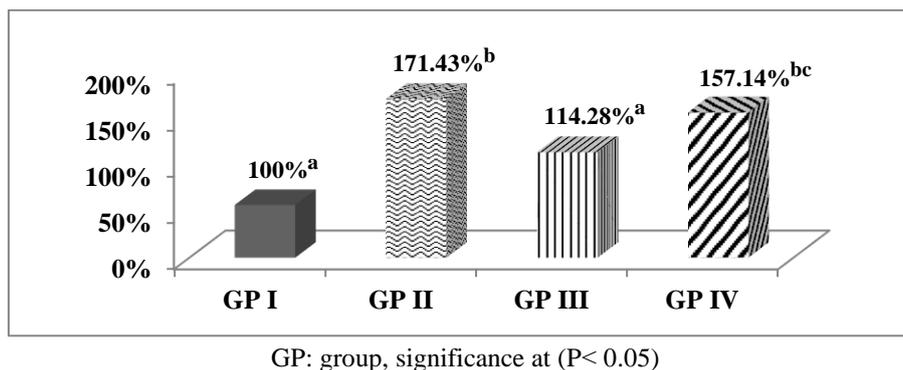


Figure 5: Fecundity rate of ewes ($n=7$) as affected by Se and PMSG (GP I) 250 IU PMSG without Se, (GP II) 250 IU PMSG with Se, (GP III) 750 IU PMSG without Se and (GP IV) 750 IU PMSG with Se.

DISCUSSION

Increasing sheep productivity by increasing twinning rate, lambing rate, litter size and fecundity is considered as an important factor in the development of Barki sheep production in Egypt. On the other hand, increasing litter size and fecundity rates in Barki sheep offer the best opportunity to increase the efficiency of lamb meat production.

Regarding to PMSG dose without Se supplementation, results in the present study indicated that, the dose of 750 IU (GP III) was found to be more effective (71.43%, 28.57%, 42.86%, 71.43% and 114.28%) as compared with 250 IU (GP I) (57.14%, 42.86%, 0.0%, 57.14% and 100%) in pregnancy rate, barren rate, twinning rate, lambing rate as well as fecundity rate especially in twinning rate which was significantly higher ($P < 0.05$) in (GP III) than that in (GP I). These results were in agreement with the previous findings of Askin (1982) who found that the pregnancy rate were found as 72.4%, multiple birth rate as 40% and litter size as 1.48 in the group treated with 750 IU PMSG, and Abdel-Mageed (2006) who reported that using one dose of PMSG (750 IU) significantly increased the ovulation rate and litter size but it had an insignificant effect on conception rate.

Gonadotropins are used to induce superovulation in sheep (Evans, 2003). So the injection of PMSG immediately after progestagen sponge removal produce an increase in the ovulation rate (Maxwell *et al.*, 1993 and Romano *et al.*, 1996). Also, Kridli and Al-Khetib (2006) reported that gonadotropins improve follicular development, increase ovulation rate and multiple births in ewes.

Improvement of reproductive performance of ewes as a result of PMSG injection may be due to the positive action of FSH in PMSG on ovarian functions. PMSG has primarily long acting FSH activity (Squires,

2003), it enhances the entry rate of small and medium follicles into larger follicles and it may prevent the occurrence of natural follicular atresia (Mandiki *et al.*, 2000), also it stimulates estradiol and progesterone secretion. In consequence, it stimulates the number of follicles, which lead to more follicular development and increase ovulation and pregnancy rates and hence enhance the twinning rate (Dogan and Nur, 2006 and Sirjani *et al.*, 2011; Rensis and López-Gatius, 2014), which leads to acceptable results on response to oestrous, fertility, and fecundity with high dose of PMSG, and can be successfully used for estrus induction and superovulation in sheep. Meanwhile, the low dose was less effective and did not increase fertility or prolificacy which was in agreement with Romano *et al.* (1996) who reported that, PMSG at 250 IU did not increase the fertility or prolificacy rates in ewes during the breeding season, and Akoz *et al.* (2006) who reported that 300 IU of PMSG did not sufficiently (42.86%) increase the multiple birth rates. That has been suggested that low PMSG doses were not sufficient to stimulate additional follicular development in ewes (Romano *et al.*, 1996), consequently reduced response to fertility or prolificacy rates. Meanwhile, it was reported that pregnancy rate in Hamadani or Akkaraman ewes which superovulated with 750 IU of PMSG was 100% (Timurkan and Yildiz, 2005 and Akoz *et al.*, 2006). Also, Koyuncu *et al.* (2001) found that when Kivircik ewes superovulated with different doses of PMSG during breeding season prolificacy rate was 196% in 700 IU group. These rates were higher than this study obtained by using 750 IU of PMSG. This difference may be explained by the influence of breed differences and some management factors on response to the treatment protocol, Karagiannidis *et al.* (2001) who reported that response to the different PMSG doses among various breeds was different.

Concerning the effect of the PMSG dose with Se supplementation, data obtained in this study indicated that pregnancy rate, twinning rate, lambing rate, as

well as fecundity rate were non-significantly higher in (GP II) (100%, 71.43%, 100%, and 1171.43%) than that in (GP IV) (85.71%, 71.43%, 85.71%, and 157.14%), and significantly higher than that in groups without Se supplementation, GP I (in all parameters) and GP III (in fecundity rate only and tend to be significantly higher in pregnancy and lambing rate), these findings were in agreement with several authors who observed a positive effect of Se administration on reproductive performance of sheep, Munoz *et al.* (2009) reported a higher conception rate, fecundity rate and a lower abortion rate in ewes treated with Se before mating. Koyuncu and Yerlikaya (2007) found that Se injection to Merino ewes significantly increased estrus response and fecundity rate (100% and 131%, respectively), Also, Balicka-Ramisz *et al.* (2006) found that Se administration prior to mating enhanced herd's reproductive success and increased the litter size by 0.38 lamb per treated ewe as compared with the control. Also, Hemingway (2003) reported that the ewes fecundity rate considerably dependent on the feed Se level. Muñoz *et al.* (2009) stated that pre-mating Se treated ewes had a higher conception rates; lower abortion rates and reared 9% more lambs than the control. Se supplementation to ewes grazing in pastures with low Se and high estrogen levels increased the conception rate, from 49 to 76% (McDowell, 1997). Also, our obtained results showed that the numbers of barren ewes were lower in PMSG plus Se supplemented groups than in PMSG without Se supplementation groups, particularly in group II (250 IU PMSG plus Se) which had no barren ewes. This finding was previously supported by Scales, (1974) who reported that pre-mating dose of 5.0 mg Se reduced the number of barren merino ewes by 15%. These results indicated that Se supplementation has a PMSG-sparing effect as the optimum dose of PMSG which give optimum reproductive performance will be reduced in Se supplemented groups. These positive effects of Se supplementation on fertility of treated females may be attributed to the increase of their serum GSH-Px level by Se supplementation, Wittwer *et al.* (2002), who found a positive correlation between the amounts of Se ingested by the animal and its serum GSH-Px level. And according to Eppig (1996), Krisher and Bavister (1998) and Livingston *et al.* (2009), stated that GSH-Px proved to be important in the maturation process of oocytes, by involving in the synthesis of biochemical components, phosphorylation of proteins and the activation of specific metabolic pathways. Also Uhm *et al.* (2007) stated that Se guarantees adequate biosynthesis of selenoprotein, protecting the embryos' cells against oxidants and apoptosis. In relation to selenoprotein, we can cite the study of Livingston *et al.* (2009), who emphasized the importance of Se in sheep reproduction in vivo, when assessing the quality of oocytes matured in vivo. On the same hand, in vitro oocyte maturation and fertilization, Hirotada *et al.* (2017) stated that Se

overcame the undesirable oxidative stress produced by hydrogen peroxide, increased blastocyst formation and enhanced the development of pre-implanted mouse embryo of mouse blastocysts cultured in the presence of Se. Also, Moraes *et al.* (2012) stated that dairy Jersey cows' diet supplemented with Se rendered the production of higher quantity of grade 1 oocytes and greater production of embryos in the process of in vitro fertilization.

The enhanced response observed in low dose PMSG 250 IU group with Se supplementation (GP II) was not found in Se-supplemented group given high dose 750 IU of PMSG (GP IV) or in groups given similar low or high doses of PMSG (250 IU or 750 IU) but without Se supplementation. Finally, our results showed that the best reproductive results were achieved by the ewes of group II (250 IU PMSG plus Se) (pregnancy rate 100 %, twinning rate 71.43 %, lambing rate 100%, as well as fecundity rate 171.43%), and also, reproductive results achieved by ewes of group IV (750 IU PMSG plus Se) were also acceptable (pregnancy rate 85.71 %, twinning rate 71.43 %, lambing rate 85.71%, as well as fecundity rate 157.14%). As the addition of high dose PMSG to an estrus synchronization program for induction of twinning tend to elevate costs, our data show a clear advantage of decreasing its dose as low as 250 IU/ewe with Se supplementation for induction of twinning and increasing of fertility and fecundity rates and appeared to be a most appropriate program because it combines simplicity and a lesser dose of gonadotropin, which also implies a reduction in cost, without reducing embryo production. However, from the practical point of view, selenium supplementation could be preferred and effectively applied with low dose of PMSG to the estrus synchronization program for induction of twinning as it showed the lowest cost.

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تأثير السيلينيوم كمكمل غذائي على تحريض إنتاج التوائم في النعاج البرقي

هویدا محمد أحمد عبد الرحمن ، مها عبد الحمید إبراهيم

E-mail: maha_doctor2013@yahoo.com Assiut University web-site: www.aun.edu.eg

هدفت هذه الدراسة إلى تقييم تأثير السيلينيوم على زيادة إنتاج التوائم للنعاج البرقي باستخدام جرعتين مختلفتين من مصل الأفراس الحوامل في برنامج تزامن الشياح خلال موسم التكاثر ، تم تقسيم 28 نعجة عشوانيا إلى أربع مجموعات ، 7 نعاج في كل مجموعة ، وتم علاج جميع النعاج باسفننج داخل المهبل ، تحتوي كل اسفنجة على 60 ملغ من خلات البروجسترون لمدة 14 يوماً ، على الفور بعد سحب الاسفننج ، تم إعطاء جرعات من هرمون مصل الأفراس كالتالي 250 وحدة دولية المجموعات الأولى والثانية و 750 وحدة دولية في المجموعتين الثالثة والرابعة في العضل.

وكانت المجموعتين الأولى والثالثة بدون مكملات إضافية فقط 0.05 ملغم سيلينيوم الموجودة اصلا في كل كغم من الغذاء و للمجموعتين الثانية والرابعة تم إضافة السيلينيوم كمكمل غذائي بنسبة 0.2 ملغم لكل كغم من الغذاء .

نتائج المجموعات الاربعة كانت على التوالي عند نسبة احتمالات 0.01%:

معدلات الحمل (71.43%، 100%، 57.14%، و 85.71%)

ونسبة النعاج التي لم تستجيب للتلقيح مطلقا (الصماء) (42.86%، 0.0%، 28.57%، و 14.29%) بالترتيب

وكان معدل حدوث التوائم (0.0%، 71.43%، 42.86%، و 71.43%) بالترتيب

وكان معدل ولادة الحولى (57.14%، 100%، 71.43%، و 85.71%) بالترتيب

ومعدل الخصوبة لنفس المجموعات هو (57.14%، 171.43%، 114.28%، و 157.14%) بالترتيب .

من نتائج التجربة يمكن استنتاج أن تقليل جرعة مصل الحوامل مع استخدام مكملات السيلينيوم أكثر ملاءمة لبرنامج زيادة إنتاج التوائم وزيادة معدلات الخصوبة .