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Effect of progestagen, PGF2α, PMSG AND GnRH on estrus synchronization and some reproductive and productive traits in Barki ewes

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Abstract

This study was carried out to investigate the effect of administration of progestagen, prostaglandin $F_2\alpha$, PMSG and GnRH on estrus synchronization and some reproductive and productive traits in Barki ewes. Increasing conception, lambing, fecundity and weaning rates and some other traits are the principle measurements for reproductive efficiency and the principle aim in the current study.This study was performed during the period from September 2012 till May 2013, in Siwa Oasis Research Station (Tegzerty Experimental Farm for animal production), belongs to Desert Research Center (DRC). This station is located at 330 Km southwest of the Mediterranean shoreline and at 65 Km east of the Libyan borders**.**

Sixty-six Barki ewes with 3-5 years old and 41.80 ± 0.559 kg average live body weight were used. Animals were randomly divided into three groups (22 ewes each). The estrous cycles of ewes in each group were synchronized using one of the following hormonal treatments: Group one (G1) received double injections of prostaglandin $F_2\alpha$ (1 ml Synchromate) 10 days apart. Ewes of group two (G2) received double injections of prostaglandin $F_2\alpha$ (1 ml Synchromate) 10 days apart and after 10 days intravaginal progestagen impregnated sponges (40 mg fluorogestone acetate, FGA) were inserted. Sponges remained in situ for 14 days. On the day of sponge removal, ewes received an intramuscular injection of 750 IU PMSG. Ewes of group three (G3) received the same treatment of group two, but one day before sponge removal ewes were injected with 2 ml (0.004 mg) of GnRH.

The results showed that, the percentage of estrus exhibition in G2 reached 95.45 % ($P<0.05$), while the lowest percentage (86.36 %) was observed in G3. The conception and lambing rates increased to 100% in G2 but there were no significant differences among experimental groups. Weaning rate was higher ($P<0.05$) for ewes in G2 than those of other groups. Litter size was higher ($P<0.05$) for ewes in G2 (1.38%) compared with other groups. The highest fecundity rate was recorded for G2 (138%) and the lowest rate was for G3 (94%). No significant differences in birth weight, weaning weight, average daily gain and mortality rate were found among groups. Gestation period was shorter ($P<0.05$) for ewes in G2 as compared with other groups. The percentage of ewes lambing twins was higher for ewes in G2 (28.57 %) than G1 (5.26), while G3 did not give twins. Moreover, G2 gave one triplet twins, while no other groups gave triplets. In conclusion, $PGF_2\alpha$ with intra-vaginal FGA sponges + PMSG regime could adequately improve the estrus synchronization and some reproductive and productive traits in Barki ewes. Treatment with $PGF_2\alpha$-FGA-GnRH was markedly inferior to the $PGF_2\alpha$-FGA- PMSG protocol in Barki ewes.

Keywords: Barki ewes, Estrus synchronization, Progestagens, Prostaglandin $F_2\alpha$, PMSG and GnRH.

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1. Introduction

Hormonal treatment to control ovulation and reproduction is a prerequisite for successful breeding and increasing the number of pregnant females [37], resulting in short breeding period and more uniform newborn crop [25]. Synchronization of estrus is a valuable management tool that has been successfully employed to enhance reproductive efficiency, particularly in ruminants [32]. In addition, the use of estrus synchronization creates the opportunity for timed breeding and lambing. This in turn results in taking advantages of seasonal variation of forage availability, photoperiod, labour resources and market demands. In small ruminant, estrus synchronization is achieved either by reducing the length of luteal phase of the estrous cycle with prostaglandin $F_{2\alpha}$ or its analogues or by extending the cycle artificially with exogenous progesterone or more potent progestagens [29,32]. Progestagens are widely used to synchronize estrus in sheep and typically result in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–80% [18]. Recent studies of Vinoles *et al*. (2001) [46]; Ataman *et al*. (2005) [7] and Zeleke *et al*. (2005) [48] have focused on the duration of the progestagen-based synchrony treatments, Intra-vaginal devices containing different types of progestagens, maintained during 6-14 days associated with or without PMSG or PMSG and $PGF_2\alpha$ combinations have been used. Intramuscular administration of 400 IU and 500-700 IU of PMSG at day when intravaginally applied sponges were removed increased the ratio of ovulation and twinning rate [11]. Other studies established that administration of GnRH at estrus can decrease variable time of LH surge and ovulation rate and it improves reproductive performance in ewes [17].

This study intended to investigate the effect of administration of progestagen, prostaglandin $F_2\alpha$, PMSG and GnRH on estrus synchronization and some reproductive and productive traits in Barki ewes.

2. Method and Methods

Location of the experiment. The present study was conducted from September 2012 to May 2013 at Siwa Oasis Research Station (Tegzerty Experimental Farm for Animal Production), which belongs to Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

Geographically, Siwa Oasis lies between longitudes 29° 06´ and 29° 24´ N and longitudes 25° 16´ and 26° 12´ E, it lies at 330 Km southwest of the Mediterranean shoreline and at 65 Km east of the Libyan borders. The ground surface height ranges from 10 to 20 meters below mean Mediterranean Sea level. Siwa Oasis is characterized by desert climate.

*Animals, housing and experimental design***.** Sixty-six non-pregnant and non-lactating Barki ewes with 3-5 years old and 41.80 ± 0.559 kg average live body weight were used. Animals were randomly divided into three groups (22 ewes each). Ewes were kept in semi-open pens roofed with wood, and were clinically healthy and free from internal and external parasites. Drinking water and mineral blocks were available all the day time. The experimental animals were fed 500 gm / head / day pelleted concentrate mixture 14 % crude protein (corn, wheat bran, unhulled cotton seed, limestone and salt) and clover hay and rice straw were provided.

The estrous cycles of the ewes in each group were synchronized using one of the following hormonal treatments: First group (G1) received double injections of prostaglandin $F_2\alpha$ (1 ml Synchromate each 1 ml solution containing 250 mg Cloprostenol, Coopers Co., Germany) 10 days apart.

On the other hand, ewes of the second group (G2) received double injections of prostaglandin $F_2\alpha$ 10 days apart, and after 10 days, intravaginal progestagen impregnated sponge (40 mg fluorogestone acetate, FGA, Chronogest®, Intervet International, B.V. Manufactured in the European Union) were inserted. Sponges remained in situ for 14 days. On the day of sponge removal, ewes received an intramuscular injection of 750 IU PMSG (Folligon, Intervet, International, B.V. Manufactured in the European Union). While, ewes of the third group (G3) were given double injections of prostaglandin $F_2\alpha$ 10 days apart and after 10 days intravaginal progestagen impregnated sponge were inserted. Sponges remained in situ for 14 days. One day before sponge removal ewes was injected intramuscularly with 2 ml, 0.004 mg, GnRH. (Receptal, Intervet International, B.V. Manufactured in the European Union).

All The experimental ewes were checked twice daily (morning and evening) to ensure that sponges remained in to place during the treatment period.

Estrus detection and mating. Three fertile Barki rams with good body conditions score were introduced to the ewes in each experimental group (one ram per 7 ewes) for estrus detection and mating; starting at the sponge withdrawal day (G2 and G3) or after second injection of $PGF_2\alpha$ (G1) for 5 consecutive days. Rams were allowed to rotate among different ewes groups to avoid sire/group confounding effect. Painted-breast fertile rams were introduced to ewes for five days. Ewes with marked rumps were considered to be mated.

The measured traits. Estrus response = number of ewes showing signs of estrus/total ewes treated x 100.

Conception rate = number of ewes conceived / number of ewes showing estrus and mated x100.

Lambing rate = number of ewes lambed / number of ewes mated.

Weaning rate = number of lambs weaned / number of lambs born.

Abortion (%) = number of ewes aborted / number of ewes conceived x100.

Litter size at birth= number of lambs born/ number of ewe lambed.

Litter size at weaning = number of lambs weaned/ number of ewe lambed.

Average litter weight at birth = the kilograms lambs born/number of ewe lambed.

Fecundity rate = number of lambs born/ number of ewe mated.

Birth weight of lambs = the kilograms lambs born/ number of lambs born.

Weaning weight = the kilograms lambs weaned/ number of lambs weaned.

Mortality rate up to weaning (%) = live lambs born-lambs weaned / live lambs born x 100.

Average daily gain (g/day) = (weaning weight – birth weight) /60.

Statistical analysis. Fecundity, litter size and mortality rates and productive traits were analyzed using General Linear Model Procedure [43]. The design was one way analysis. The model was as follows:

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

Where, Y_{ij} = any observation of j^{th} animal within i^{th} treatment;

μ = overall mean;

T_i = effect of i^{th} treatment (i = 1-3);

e_{ij} = experimental error.

Duncan Multiple Range Test [13] was used to test the level of significance among means.

While, response to estrus synchronization, fertility traits, type of lambing and sex ratio were analyzed using Chi-square test.

3.Results and discussion

1- Response to estrus synchronization. No ewe lost an intravaginal sponge during the treatment period. Response to estrus synchronization, conception rate, lambing rate, weaning rate and abortion are presented in Table 1. There were significant differences in the estrus response and weaning rate between the G2 and G3. While, there were no significant differences in conception, lambing and abortion rates among the three groups. From the 66 Barki ewes used in the trial, 60 ewes exhibited signs of estrus during the observation period (120 hours), the second group showed significant ($P<0.05$) increase of ewes responded to estrus synchronization followed by G1 then G3, whereas no significant difference was observed between the first and third groups. The higher estrus response of Barki ewes treated with $PGF_2\alpha$, FGA impregnated sponges + PMSG (G2) may be due to the fact that progestagen prevents new corpora lutea formation and PMSG improves the synchronization. Progestagens and $PGF_2\alpha$ or their analogues are generally used in order to condense parturition and estrus of the ewes in the breeding season. Hormones such as GnRH, PMSG, FSH, and LH may be used to increase pregnancy rate and number of lambs [36]. The response to estrus synchronization obtained in the present work on Barki ewes was higher than the estimates of Mansour *et. al*. (1993) (77%) [34], Lethy *et. al.* (2003) (85%)

[33], Abdel-Mageed (2006) (84%) [1] and Abdullah *et. al*. (2002) (80.9%)[2]. And lower than the 100% reported in Karakul ewes [23], 100% reported in Akkaraman crossbred ewes [6]. The estrus response rate was similar to the findings of the previous studies by Nasroallah *et al.* (2012) [39] who found that estrus response was 93.3% when used protocol $PGF_2\alpha$–FGA–eCG, but report that decreased in estrus response was 71.4% when used protocol $PGF_2\alpha$–FGA– GnRH. Whereas in the current study, $PGF_2\alpha$–FGA–GnRH (G3) treatment resulted in the lowest percentages of females coming into estrus after natural service. The estrus response rate was similar to the findings of the previous studies by Koyuncu and Alticekic (2010) [30] who found that estrus was induced in 96% of the ewes.

Occurrence of estrus in ewes treated with either synthetic progestagen or natural progesterone in conjunction with PMSG varied from 47% [41] to 80% or greater [24]. So far, a high estrus response (75-100%) has been recorded with the FGA-PMSG [38,40]. The absence of estrus may be due to inadequate estradiol secretion by the ovarian follicles, indicating incomplete follicular growth and development [8].

The PMSG has been shown to reduce the interval from sponge withdrawal to estrus and improve the efficiency of synchronization of estrus and ovulation during the breeding season.

2 - Conception rate. The conception rate was relatively higher (100%) in G2 and G3 than G1 (95%) but, with no significant differences. This result was higher than that obtained by Hussein et al. (2003) [27] who found that Rahmani ewes treated with 400 IU PMSG showed conception rate of 70 %, 72% obtained by Abdel-Mageed (2006) [1]. This result was almost agreed with those reported by Akoz, *et. al*., (2006) [6] who reported that conception rate in Akkaraman crossbred ewes treated with FGA+700 IU PMSG was 100%. Pregnancy rate was in agreement with that observed by Huseyin and Yildiz, (2005) [26] who reported that pregnancy rate in ewes treated with FGA + 750 IU of PMSG was 100%. Gulyuz and Kozat (1995) [22] found that ewes treated with 40 mg FGA+750 IU PMSG the percentage of pregnancy rate was 96%**.**

It was pointed out that administration of PMSG increased the number of follicles and therefore raised the twinning and triplet rates [22]. To enhance the estrus response and pregnancy rate after progestagen treatment during breeding season, luteal regression with a $PGF_2\alpha$ injection should be ensured [35]. This discrepancy in the results reported by different researchers on pregnancy and estrus response rates can be explained by the differences in body condition, breed, and management systems. It was reported that percentage of estrus and pregnancy rates in ewes treated with 30 mg of Cronolone (FGA) +700 IU of PMSG were 90% and 76.4% respectively [45].

3 - Lambing rate. Lambing rate was ranged between 94.74 to 100 %, and was higher in G2 than other groups, but with no significant differences. This result was higher than 75.6% [30]**,** 80% [47] and 90% reported by Crosby and O'Callaghan (1991) [10] in ewes fitted with intravaginal sponges followed by PMSG injection. Akoz, et al, (2006) [6] reported that lambing rate in Akkaraman crossbred ewes treated with FGA+700 IU PMSG was 85.7%. Kulaksiz et al. (2013) [31] found that lambing rate was higher in group treated with FGA-PMSG (93.3%), but was reduced to 50% when used a protocol of FGA-GnRH-$PGF_2\alpha$. Lambing rate in our study was found to be higher compared with normal range of lambing rate for Barki sheep (84.6 to 92%) as obtained previously by Abol-Naga and Aboul-Ela (1986) [4,5]. The increase in lambing rate in the second group may reflect the higher incidence of ovulation rate. Ibrahim (1993) [28] reported that the effect of PMSG in enhancing fertility is probably a direct consequence of its action in increasing ovulation rate. In this study the higher pregnancy and lambing rates observed in the $PGF_2\alpha$ -FGA-PMSG group may be due to the PMSG action. Also the lambing rate was highly dependent on the ewe breed [16], progestagen treatment protocol [12], the breeding season [7,48] and the AI procedure [35].

4 - Abortion rate. As mentioned in the table 1, no significant differences among the groups were observed concerning the abortion rate. Both G1 and G2 showed no abortion, while, G3 showed 5.26% abortion. This result was similar to finding obtained by Abdel-Megeed (2006) [1] in Rahmani ewes treated with 500 IU PMSG. The estimate of aborted ewes of G1 in the present work is lower than 5.2%

recorded by Farrag et al. (2010) [19] in Barki ewes treated with two doses of $PGF_2\alpha$, 11 days intervals.

5- *Weaning rate.* Weaning rate was ranged between 83.33 to 89.66 %, with higher significant values in G2 than other groups.

Weaning rate of G1 in the present work is close to 90% that recorded by Farrag et al. (2010) [19] in Barki ewes treated with two doses of $PGF_2\alpha$, but weaning rate of G2 in the present work is lower than 100% that recorded by Farrag et al. (2010) [19] in Barki ewes treated with 1000 IU of PMSG.

Table 1. Response to estrus synchronization and fertility traits (LSM±SE) of Barki ewes as affected by $PGF_2\alpha$, FGA and PMSG (G2) and $PGF_2\alpha$, FGA and GnRH (G3) compared to the control (G1)

Traits	G1	G2	G3	Ovarall mean
RTES*	90.91^{ab}±4.04 (20/22)	95.45^{a}±4.04 (21/22)	86.36^{b}±4.04 (19/22)	90.91±4.04
Conception rate	95.00^{a}±4.33 (19/20)	100.00^{a}±4.33 (21/21)	100.00^{a}±4.33 (19/19)	98.33±4.33
Lambing rate	95.00^{a}±4.19 (19/20)	100.00^{a}±4.19 (21/21)	94.74^{a}±4.19 (18/19)	96.67±4.19
Weaning rate	85.00^{ab}±2.95 (17/20)	89.66^{a}±2.95 (26/29)	83.33^{b}±2.95 (15/18)	86.57±2.95
Abortion rate	0.00^{a}±4.26 (0/19)	0.00^{a}±4.26 (0/21)	5.26^{a}±4.26 (1/19)	1.69±4.26

Means within each row with different superscripts are significantly different at 5% level.
*RTES= Response to estrus synchronization

Table 2. Fecundity rate and litter size at birth and weaning (LSM±SE) of Barki ewes as affected by different hormonal treatments, compared to control

Traits	G1	G2	G3	Overall mean
Fecundity rate	1.00^{b}±0.09 (20/20)	1.38^{a}±0.09 (29/21)	0.94^{b}±0.09 (18/19)	1.15±0.05
Litter size at birth	1.05^{b}±0.08 (20/19)	1.38^{a}±0.08 (29/21)	1.00^{b}±0.08 (18/18)	1.11±0.05
Litter size at weaning	0.89^{b}±0.12 (17/19)	1.23^{a}±0.11 (26/21)	0.83^{b}±0.12 (15/18)	1.00±0.07

Means within each row with different superscripts are significantly different at 5% level

Table 3. Some productive traits (LSM±SE) of Barki ewes as affected by $PGF_2\alpha$, FGA and PMSG (G2) and $PGF_2\alpha$, FGA and GnRH (G3) compared to the control (G1)

Traits	G1	G2	G3	Overall mean
Birth weight (kg) (Lambs N)	3.34^{a} ±0.16 (20)	3.01^{a} ±0.14 (29)	3.27^{a}±0.17 (18)	3.18±0.09
Litter weight at birth (Ewes N)	3.51^{ab}±0.28 (19)	4.16^{a}±0.27 (21)	3.27^{b}±0.29 (18)	3.67±0.16
Mortality rate	15.00^{a}±8.19	10.34^{a}±5.75	16.66^{a} ±9.03	13.43±4.19
Weaning weight (Kg) (Lambs N)	13.21^{a} ±0.33 (17)	12.55^{a} ±0.31 (26)	13.24^{a} ±0.20 (15)	12.92±0.18
Av. daily gain (g)	163.62^{a}±5.69	158.14^{a}±4.02	166.44^{a}±3.19	161.89±2.59

Means within rows with different superscripts are significantly different at 5% level.

6 - Fecundity and litter size at birth and weaning. Fecundity and litter size at birth and weaning in Barki ewes are shown in Table 2. Increasing Barki sheep productivity by increasing lambing rate, litter size and fecundity is considered an important factor in the development of Barki sheep production in Egypt. On the other hand, increasing litter size and fecundity rates in sheep offers the best opportunity to increase the efficiency of lamb meat production. In the present study, there were significant increases in fecundity and litter size at birth and weaning in the second group as compared to the other groups. Fecundity rates recorded following estrus synchronization were found to be 100, 138 and 94% in the first, second and third groups, respectively ($P < 0.05$). The fecundity rate was similar to the findings of the previous studies by Zarkawi et al. (1999), [47] who reported that fecundity rate was 137.5% when used protocol MAP + 600 IU of PMSG in Awassi ewes. But, was higher than the findings of Koyuncu and Alticekic (2010) [30], who found that fecundity rate was 114.6% when used technique FGA +PMSG in Kivircik ewes. Safdarian et al. (2006) [42] found that fecundity rate was 133% when used protocol MAP + 500 IU of PMSG and 75% when used protocol $PGF_2\alpha$- $PGF_2\alpha$ in Karakul ewes. The result in the third group was lower than that obtained by Titi et al. (2010) [44] who found that combination of GnRH, progestagen sponges and $PGF_2\alpha$ can be effective in synchronizing estrus and improving fecundity in sheep and goats.

Litter size at birth is expressed as number of born lambs per ewe lambed and is considered an important parameter of fertility. The mean litter size in groups 1, 2, and 3 were 1.05, 1.38 and 1.00, respectively. This result was in agreement with many other investigations on Rahmani ewes that showed that litter size increased as a result of treating with PMSG [1,25]. Safdarian et al. (2006) [42] reported that litter size was 1.4% when used MAP + 500 IU of PMSG protocol and 1.25% when used $PGF_2\alpha$- $PGF_2\alpha$ protocol in Karakul ewes.

The litter size obtained in G2 (1.38) is in agreement with the findings of Zeleke et al. (2005) [48] who stated that the mean litter size when used progestagen and PMSG in Dorper ewes was 1.30. Litter size in G1 (105%) was similar to the findings of the previous studies by Galal (1987) [20,21], which was 104% in Barki sheep. These results agreed with those reported by Ibrahim (1993) [28] who found that the number of lambs born per ewe lambed (litter size) was greater in ewes received PMSG to induce super ovulation than in non treated ones. Litter size at weaning in groups 1, 2, and 3 were 0.89, 1.23 and 0.83%, respectively. Litter size at weaning in group 2 was higher significantly than in the other groups ($P<0.05$), however, there were no significant differences between the first and third groups. The result in G1 (0.89) was similar to the findings of the previous studies by Abdel-Megeed (2006) [1] in Rahmani ewes treated with $PGF_2\alpha$, but when using 500 IU of PMSG litter size at weaning was 1.33. The results indicated that the treatment of PMSG increased litter size at weaning.

7 - Some productive traits. From Table (3), there were no significant differences among the experimental groups in birth weight, mortality rate, weaning weight and average daily gain, but there were significant differences in litter weight at birth. All groups in this study had almost the same birth weight being 3.34, 3.01 and 3.27kg, for G1, G2 and G3, respectively. These results were in agreement with the result found by Abdel-Megeed (2006) [1] who reported no significant differences between treated and untreated groups with PMSG. Our results of birth weight were higher than those obtained in Barki sheep (2.3, 2.4 kg) according to El-Kouni et al. (1974) [15] and El-Kimary (1975) [14], but close to those obtained by Barghout and Abdel-Aziz (1986) [9] (3.20 kg), and slightly lower than those reported by Lethy et al. (2003) [33] (3.52 kg).

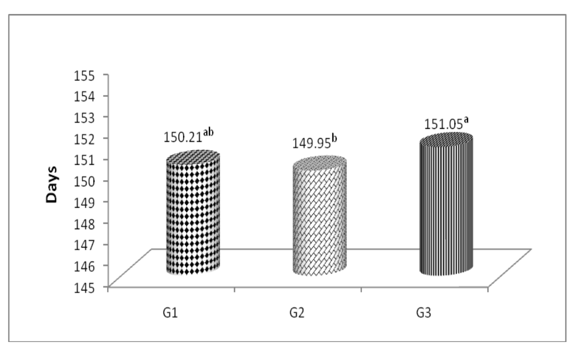

Figure 1. Gestation length of Barki ewes as affected by $PGF_2\alpha$, FGA and PMSG (G2) and $PGF_2\alpha$, FGA and GnRH (G3) compared to the control (G1)

Treatment of ewes with PMSG (G2) increased litter birth weight ($P<0.05$). This result was in agreement with the result found by Abdel-Megeed (2006) [1] in Rahmani ewes treated with $PGF_2\alpha$ (3.68) and PMSG (4.52).

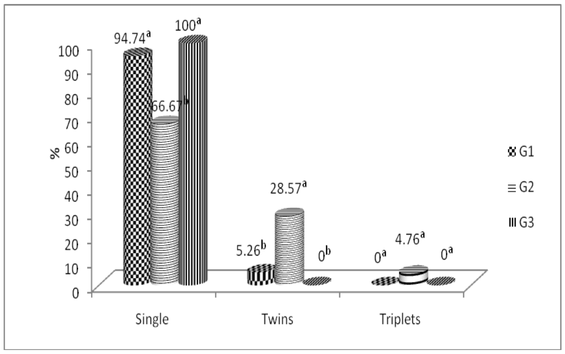

Figure 2. Type of lambing of Barki ewes as affected by $PGF_2\alpha$, FGA and PMSG (G2) and $PGF_2\alpha$, FGA and GnRH (G3) treatment groups compared to the control (G1)

These results are in agreement with those reported by Ibrahim (1993) [28] and Aboul-Ella (2006) [3]. There was no significant difference in the percentage of ewes lambing single in the three groups.

From (Figure 3), there was no significant difference in sex ratio of lambs among the three groups, being 40, 44.83 and 50% males for G1, G2 and G3, respectively. Their counterparts of females percentage were 60, 55.17 and 50%, respectively.

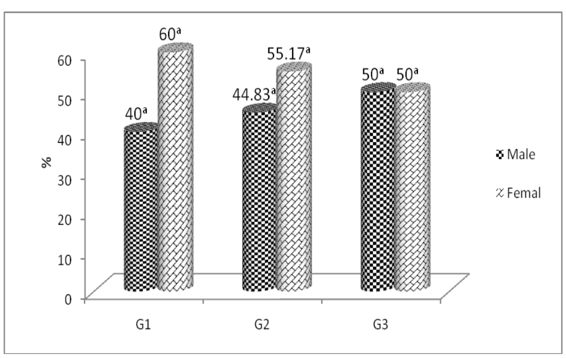

Figure 3. Sex ratio of Barki ewes as affected by $PGF_2\alpha$, FGA and PMSG (G2) and $PGF_2\alpha$, FGA and GnRH (G3) compared to the control (G1)

4.Conclusion

In general, Barki is an excellent breed of sheep if well managed and selected. Results of the trial indicated that reproductive performance of Barki sheep can be improved by applying estrus synchronization and super ovulation practices despite living under the harsh conditions. Barki ewes were capable of being bred and lambing using different estrus synchronization protocols. $PGF_2\alpha$, FGA sponge and PMSG protocol to Barki ewes appear to be more effective in the synchronization of estrus, fertility and fecundity rates and litter size than other protocols. In this respect, we suggest some modifications (such as studying the durations between GnRH and $PGF_2\alpha$ administrations) of synchronization protocols to achieve further improvements in the ultimate fertility.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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