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RESEARCH ARTICLE

The Combined Effect of Prostaglandin Administration and Ram Introduction in Multiparous and Nulliparous Sheep in Anestrous Period on Prolificacy [1]

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Summary

In the study it was aimed to investigate and compare the combined effectiveness of ram introduction (ram effect) and prostaglandin F2 alpha (PGF2α) administration in multiparous and nulliparous Kangal White Karaman ewes during the out of breeding season. The ewes were first divided into two main groups: non-lactating multiparous (Group M, n=104) and nulliparous (Group N, n=101). The multiparous and nulliparous animals were further divided in to two subgroups. Group MRP (n=50 multiparous) and NRP (n=51 nulliparous) were injected with a single dose of PGF2α on the first day of ram introduction. And the Group MR (n= 54) and NR (n= 50) served as controls with ram introduction but no PGF2α injection. In all of the groups, adult, purebred and fertile rams stayed with the ewes for 45 days. The blood samples were collected at 3-day intervals for 18 days after ram introduction from subsets of ewes (n=17 per group) to monitor the serum progesterone concentration. The total lambing ratios in multiparous and nulliparous animals were 72.1% (75/104) and 44.6% (45/101), respectively (P<0.001). Among the PGF2α and non-PGF2α subgroups of multiparous and nulliparous ewes, the lowest lambing rate was observed in Group NR (36.0%). In multiparous ewes (Groups MR and MRP), the mean progesterone level varied significantly among the days (P<0.001). In contrast, in Group NRP, the progesterone levels varied significantly over the tested time course (P<0.001), but no differences were detected in Group NR (P>0.05). We concluded that being multiparous contributes to the success of PGF2α administration in combination with ram introduction in the anestrous period in ewes. Furthermore, PGF2α administration together with ram introduction positively affects the lambing rate in nulliparous ewes.

Keywords: *Ram effect, Prostaglandin F2 alpha, Multiparous, Nulliparous Kangal White Karaman, Ewe, Out of breeding season*

Anöstrus Dönemindeki Multipar ve Nullipar Koyunlarda Koç Etkisi ve Prostaglandin Uygulamalarının Dölverimi Üzerine Kombine Etkisi

Özet

Bu çalışmada, üreme sezonu dışında multipar ve nullipar Kangal Akkaraman ırkı koyunlarda koç katımı (koç etkisi) ve prostaglandin F2α (PGF2α) uygulaması kombinasyonunun etkinliğinin karşılaştırılması ve araştırılması amaçlandı. Koyunlar öncelikle, laktasyonda olmayan multipar (n=104, Grup M) ve nullipar (n=101, Grup N) koyunlar olmak üzere iki ana gruba ayrıldı. Daha sonra multipar ve nullipar koyunlar iki alt gruba ayrıldı. Grup MRP (n=50 multipar) ve Grup NRP'ye (n=51 nullipar) koç katımının ilk günü, tek doz PGF2α enjekte edildi. Grup MR (n= 54) and NR (n= 50) ise PGF2α enjekte edilmeden sadece koç katımı yapılarak kontrol grubu olarak seçildi. Tüm gruplarda fertil, ergin ve safkan koçlar 45 gün süre ile sürüde kaldı. Kan progesteron düzeylerinin izlenmesi amacıyla her bir grup için oluşturulan subsetgruplardan (n= 17) 18 gün boyunca, üç gün aralıklarla kan alındı. Multipar ve nullipar hayvanlarda total kuzulama oranları sırasıyla %72.1 (75/104) ve %44.6 (45/101) olarak belirlendi (P<0.001). Multipar ve nullipar koyunlarda PGF2α uygulanan ve uygulanmayan alt gruplarda %36.0 kuzulama oranı ile en düşük sonuç Grup NR'de belirlendi (P<0.001). Multipar koyunlarda (Grup MR ve Grup MRP) günler arasındaki ortalama progesteron düzeyleri arası fark önemli bulundu (P<0.001). Ancak nullipar koyunlarda günler arası progesteron düzeyi Grup NRP'de önemli iken (P<0.001), Grup NR'de önemsiz bulundu (P>0.05). Sonuç olarak; anöstrus döneminde koç katımı ve PG F2α kombinasyonu uygulamalarında multipar olmanın etkili olduğu, bununla birlikte nullipar koyunlarda koç katımı ile birlikte PGF2α uygulamasının kuzulama oranını artırdığı tespit edildi.

Anahtar sözcükler: *Koç etkisi, Prostaglandin F2 alfa, Multipar, Nullipar Kangal Akkaraman, Koyun, Üreme mevsimi dışı*

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INTRODUCTION

Kangal White Karaman sheep represent a local and national breed that was registered in 2012 and is characterized by adaptation to the hard environmental conditions found in Turkey [1,2]. In northern hemisphere countries such as Turkey, sheep usually show a seasonal sexual cycle. The breeding season usually begins at the end of August and continues to the end of November [3,4].

Since sheep breeding is naturally seasonal, prolificacy is restricted temporally. Therefore, expanding the application of reproductive biotechnologies such as estrous and ovulation synchronization that can be performed in and/or out of breeding season is essential to increase prolificacy and market availability and provide economically significant and controlled breeding and genetic advancement [5]. In addition, these types of applications should not result in additional costs for the breeder or harm the environment, animal welfare or public health.

When rams are introduced into a herd of ewes during the anestrous period, the sexual cycle is stimulated and copulation, ovulation and pregnancy occur; this is defined as the 'ram effect' [6-8]. The ram effect has been well established [5,9]. Introducing rams into a sheep herd out of breeding season to stimulate estrous is one of the easiest, most economical and environmentally friendly synchronization methods that does not compromise animal welfare or public health. Therefore, its use has become widespread [10-13]. However, new applications are needed to increase the effectiveness of the ram effect.

Sudden introduction of rams during the anestrous period stimulates the release of luteinizing hormone (LH) [14-16]. The time between ram introduction and LH release varies between 20 and 30 hours [7,17]. Applications that arrange the luteal phase would be helpful in increasing the effectiveness of estrous. Ungerfeld and Rubianes [18] reported that estrous signs were more prominent in sheep that received medroxyprogesterone prior to ram introduction than in untreated sheep; in the early period of the breeding season, increased follicular growth rates and rising estrogen concentrations led to increased LH release. However, steroids have negative effects on the environment and human and animal health; thus, the administration of prostaglandin F2 alpha (PGF2α) instead of steroids would be beneficial [13,19]. Therefore, studies have been conducted to find effective methods of using PGF2α administration together with the ram effect, particularly in sheep herds in out of breeding season [20].

Varying results have been reported regarding the effects of PGF2α administration on estrous synchronization in and out of breeding season [15,21-23].

The aim of this study was to test the following hypotheses: 1) The combination of ram introduction (ram effect) and PGF2α administration stimulates cycle activities better then only ram effect in out of breeding season, 2) Nulliparous and multiparous animals could have different responses to these applications.

MATERIAL and METHODS

In the study, 205 Kangal White Karaman ewes in the anestrous period (101 nulliparous and 104 non-lactating multiparous) and 20 fertile rams of the same breed bred by a family company in the Sivas province were used. The animals grazed on native pasture during the study and were kept in the same yard at night. The study was conducted during May and June, in out of breeding season [3].

The ewes included in the study were initially divided into two main groups: Group M (multiparous, n=104) and Group N (nulliparous, n=101). Subsequently, each group was divided into two subgroups (Group MR, MRP and Group NR, NRP). The animals in Group MRP (n=50 multiparous) and Group NRP (n=51 nulliparous) were injected with a single dose of 125 µg cloprostenol (PGS®, Alke, Turkey) via the intramuscular (IM) route on the first day of ram introduction. Groups MR (n=54 multiparous) and NR (n=50 nulliparous) served as controls and were not administered cloprostenol. After being separated for 2 months, all of the ewes were exposed to 20 rams for 45 days.

Seventeen animals were randomly selected from each group, and subsets were created to measure the progesterone concentrations. Blood was obtained from the vena jugularis on days 0, 3, 6, 9, 12, 15 and 18 of ram introduction. The serum samples were separated and stored at -20°C until all the samples had been collected. The progesterone levels were then determined using a commercial ELISA kit (Catalogue No: EIA 1561, DRG International Inc., Marburg, Germany) and an ELISA reader (Bio Tek Instruments, USA) [24].

SPSS 14.01 software was used for the statistical analyses. Variance analysis was used to compare the mean progesterone levels on different days. Student's t-test was used to compare the progesterone levels between groups on a given day. A chi-square test was used to compare the lambing ratios between groups and cyclic conditions at the beginning of the study. Differences were considered to be significant when $P<0.05$.

Ethics committee approval was obtained from the Animal Tests Ethics Committee, Firat University (FÜHADEK, 2011/11-138).

RESULTS

The total lambing ratio was 72.1% (75/104) for the multiparous animals and 44.6% (45/101) for the nulliparous animals; the difference found significant ($P<0.001$). When

the lambing ratios were compared among the four groups (NRP, NR, MRP and MR), the lowest lambing ratio was detected in Group NR (18/50, 36%) (P<0.05). There was no significant difference between the lambing ratio in subgroups of multiparous and nulliparous ewes (MRP and MR/NRP and NR) *(Table 1)*.

The mean progesterone concentrations were greater than 1 ng/ml in both the nulliparous and multiparous animals on the first day of ram introduction *(Table 2, Table 3)*. Also the luteal and follicular phase ratios were found similar in all groups (P>0.05).

Comparisons of the progesterone concentrations on different days in inter-groups without being multiparous and nulliparous, significant differences were detected between the PGF2α and non- PGF2α groups (P<0.001). While the differences in the mean progesterone concentrations on different days were significant in the Groups MRP, MR and NRP (P<0.001), no such differences were found in the nulliparous control ewes (NR) (P>0.05).

DISCUSSION

The Kangal White Karaman breed of sheep is a local breed, registered in 2012. It is considered a seasonal polyestrous breed. Although the breeding season is primarily in October and November, it is defined as autumn and the beginning of winter [1,4]. This study was conducted in May and June, the out of breeding season [3].

In recent years, methods such as the introduction of rams have been used to induce sheep breeding because they have fewer pharmacologic side effects, are more environmentally friendly, and pose less risk to animal welfare, public and human health. Increasing the effectiveness of these methods outside of the breeding season would be economically beneficial to the sheep industry [25,26]. Although long-term use of progesterone to increase breeding efficiency raises concerns due to the harmful effects on the environment and human and animal health, PGF2α is more environmentally friendly and poses less

Table 1. *The lambing ratios in nulliparous and multiparous ewes*
Tablo 1. *Nullipar ve multipar koyunlarda kuzulama oranı*

Animal	**Multiparous (n=104)**		**Nulliparous (n=101)**		**P**
Application	**PGF (+) (Group MRP)**	**PGF (-) (Group MR)**	**PGF (+) (Group NRP)**	**PGF (-) (Group NR)**	
n	54	50	51	50	
Lambing ratios (%)	37/54 (74.0)^{a}	38/50 (70.4)^{ac}	27/51 (52.9)^{bc}	18/50 (36.0)^{b}	*
Total (%)	75/104 (72.1)		45/101 (44.6)		*

PGF (+): *cloprostenol injection,* ***PGF (-):*** *no cloprostenol;* ^{a,b,c} *Numbers/percentages with different superscripts within a row differ significantly; * P<0.001*

Table 2. *Comparison of the mean progesterone concentrations (ng/ml) (± standard deviation) in multiparous ewes at 3-day intervals*
Tablo 2. *Multipar koyunlarda 3 gün aralıklarla ortalama progesteron (ng/ml) (± standard hata) konsantrasyonlarının karşılaştırılması*

Groups	**Days (X±Sx)**							
	0	**3**	**6**	**9**	**12**	**15**	**18**	**P**
Group MRP	1.02±0.17^{a}	0.91±0.25^{a}	2.42±0.32^{b}	3.46±0.86^{b}	4.25±0.77^{b}	5.36±1.04^{b}	4.15±1.16^{b}	*
Group MR	1.41±0.44^{a}	0.71±0.15^{a}	2.95±0.57^{b}	4.21±0.70^{b}	4.66±0.63^{b}	4.34±0.66^{b}	3.58±1.12^{b}	*
P	-	-	-	-	-	-	-	

^{a,b} *Values with different superscripts within a row differ significantly; - P>0.05; * P<0.001*

Table 3. *Comparison of the mean progesterone concentrations (ng/ml) (± standard deviation) in nulliparous ewes at 3-day intervals*
Tablo 3. *Nullipar koyunlarda 3 gün aralıklarla ortalama progesteron (ng/ml) (± standard hata) konsantrasyonlarının karşılaştırılması*

Groups	**Days (X±Sx)**							
	0	**3**	**6**	**9**	**12**	**15**	**18**	**P**
Group NRP	1.70±0.41^{a}	1.28±0.29^{a}	2.66±0.45^{b}	2.69±0.54^{b}	3.72±0.58^{b}	4.03±0.80^{b}	2.17±0.57^{b}	*
Group NR	1.85±0.39	1.59±0.45	3.05±0.58	2.58±0.42	3.30±0.64	3.14±0.80	3.36±0.97	-
P	-	-	-	-	-	-	-	

^{a,b} *Values with different superscripts within a row differ significantly; - P>0.05; * P<0.001*

health risks than progesterone. However, the low efficiency obtained with PGF2α use in and/or out of breeding season has compelled researchers to improve such methods. In the present study, a single dose of PGF2α was used as an alternative to a double dose. The aim of the administration of a single dose of PGF2α combined with ram introduction was to increase the ram effect-induced ovulation ratios [22,26]. The results obtained from the study demonstrate that this goal was achieved, particularly in nulliparous sheep.

The inadequate results obtained from estrous synchronization with synthetic prostaglandin analogues and artificial insemination may be explained by corpus luteum dysfunctions due to insufficient hormones, early embryonic death, implantation disorders, late embryonic death and/or fetal losses [23]. In contrast, it has been reported that estrogen receptor alpha and progesterone receptor protein expression levels are decreased in oviductal and uterine cells in response to progesterone compared with prostaglandin in synchronization studies [27]. However, the variability in ovulation time after PGF2α application may be minimized by the ram effect. The ram effect is commonly used for LH pulsation and ovulation induction during seasonal anestrous in ewes [16]. This effect is also observed in sheep that are administered progesterone [28] and PGF2α [5]. Thus, the administration of PGF2α combined with the ram effect may be of benefit during the early luteal phase to provide fixed-time artificial insemination without estrous detection in ewes [29]. There is little or no knowledge regarding the use of PGF2α administration to stimulate cycles on the first day of ram introduction in ewes in the anestrous period.

The corpus luteum formed after male-induced ovulation has either a normal life span (luteal phase lasting 18-19 days) or regresses early (5-6 days). An ovulatory subestrous period may follow this period. The progesterone levels detected in the present study are consistent with those reported in the literature [7,30,31]. When the reduction in progesterone concentration is compared with natural luteolysis, it is more prominent after PGF2α-induced luteolysis. Although luteal regression after natural PGF2α release lasts for 72 h, after exogenous PGF2α administration, it lasts for only 6-24 h [32]. In the present study, for multiparous and nulliparous anestrous ewes, on the first day of ram introduction, no difference in the progesterone levels was observed between the PGF2α-treated sheep and controls *(Table 2, Table 3)*.

Based on the progesterone levels measured at the beginning of the study (day 0), 19/34 (55%) nulliparous animals and 17/34 (50%) multiparous animals were found to be in the luteal phase (P>0.05). In a similar study, Smith et al.[20] found that 14% of progesterone-treated ewes were in the luteal phase at the first day of application. The difference may be attributed to the breed of sheep and the location.

In the present study, serum progesterone levels in multiparous and nulliparous ewes were greater than 1 ng/ml on the first day of ram introduction, demonstrating that at least partial cyclic activities are present during the anestrous period in Kangal White Karaman ewes. Consistent with the results presented in this study, Kaulfuss et al.[33] detected 100% cyclic luteal structures in ewes in breeding season with no differences noted between breeds. However, depending on the breed, they observed 10-60% cyclic luteal structures during the rest of the year. Similarly, in a study by Maatoug-Ouzini et al.[34] using Barbarina sheep, the different cycle types formed after ram introduction were analyzed. They found that 30% of the Barbarina sheep continued cyclic activities out of breeding season, and this rate reached 60% with the ram effect.

In the present study, both nulliparous and multiparous sheep were found to respond to the ram effect. Nevertheless, the lambing ratios were significantly higher in the PGF2α-administered groups on the first day of ram introduction *(Table 1)*. In addition, it was found that PGF2α administration on the first day of ram introduction was more effective in the nulliparous animals than multiparous animals. Also when we compared the progesterone levels between days, multiparous animals were more affected by the ram introduction instead of PGF2α application. Nonetheless, in nulliparous animals PGF2α administration with ram introduction found more effective.

In our study, the lambing rates of the multiparous and nulliparous ewes, 72.1% and 44.6%, respectively, were significantly different (P<0.001). The difference is most likely due to ovulation insufficiency and high pre-implantation losses in the nulliparous animals. Supporting this assumption, Khan et al.[35] reported the embryonic loss rate as 20-40% in adults and 50-63% in young ewes. In a review by Rosa and Bryant [4], it was reported that ovulation in multiparous sheep was approximately three-fold greater than in 14- to 15-month-old nulliparous sheep when using the ram effect.

In a study conducted by Reyna et al.[36] in Australia, the pregnancy rates achieved with fixed-time insemination were 11-32% in different groups of multiparous and nulliparous animals out of breeding season. These rates are lower than those observed in the present study. The low pregnancy rates detected in similar studies conducted both in and out of breeding season may be attributed to factors such as artificial estrous or ovulation induction, different concentration or type of sperm, fixed-time inseminations and different techniques [23,37,38]. The higher lambing ratios obtained in the present study may due to the continuous presence of rams with the ewes until mating [39].

It is also important to emphasize that the progesterone values measured in this study demonstrate that nulliparous ewes respond more strongly to ram introduction when treated with PGF2α than multiparous ewes. Thus, being

nulliparous must be considered a negative factor for breeding in normal situations.

The results of the current study suggest that cyclic activities continue to some degree throughout the year in Kangal White Karaman sheep. Multiparous Kangal White Karaman sheep in anestrous period responded better to the combined PGF2α administration and ram effect than nulliparous ewes. PGF2α administration at the time of ram introduction increased the lambing ratio in nulliparous sheep. Further studies are required to determine the cycle-induction mechanism of PGF2α administration and ram introduction and to assess their impact on ovulation.

REFERENCES

1. Yilmaz O, Denk H, Bayram D: Effects of lambing season sex and birth type on growth performance in Norduz lambs. *Small Rumin Res*, 68, 336-339, 2007.

2. Official Gazette: Yerli hayvan ırk ve hatlarının tescili hakkında tebliğde değişiklik yapılmasına dair tebliğ (Tebliğ No: 2012/61). *Turkish Official Gazette*, 28384, 14 August, 2012.

3. Uyar A, Alan M: Koyunlarda erken anöstrus döneminde melatonin uygulamalarının ovulasyon ve gebelik üzerine etkisi. *YYÜ Vet Fak Derg*, 19 (1): 47-54, 2008.

4. Rosa HJD, Bryant MJ: Seasonality of reproduction in sheep. *Small Ruminant Res*, 48, 155-171, 2003.

5. Contreras-Solis I, Vasquez B, Diaz T, Letelier C, Lopez-Sebastian A, Gonzalez-Bulnes A: Ovarian and endocrine responses in tropical sheep treated with reduced doses of cloprostenol. *Anim Reprod Sci*, 114, 384-392, 2009.

6. Ungerfeld R, Forsberg M, Rubianes E: Overview of the response of anoestrous ewes to the ram effect. *Reprod Fert Develop*, 16, 479-490, 2004.

7. Chemineau P, Pellicer-Rubio MT, Lassoued N, Khaldi G, Monniaux D: Male-induced short oestrous and ovarian cycles in sheep and goats: A working hypothesis. *Reprod Nutr Dev*, 46, 417-429, 2006.

8. Delgadillo JA, Gelez H, Ungerfeld R, Hawken PAR, Martin GB: Revisiting the dogmas surrounding the mechanisms involved in the male effect in sheep and goats. *Behav Brain Res*, 200, 304-314, 2009.

9. Underwood EJ, Shier FL, Davenport NJ: The breeding season of Merino, crossbred and British breed ewes in the agricultural districts. *J Agric*, 11, 135-143, 1944.

10. Hawken PAR, Beard AP: Ram novelty and the duration of ram exposure affects the distribution of mating in ewes exposed to rams during the transition into the breeding season. *Anim Reprod Sci*, 111, 249-260, 2009.

11. Martin GB, Scaramuzzi RJ, Lindsay DR: Effect of the introduction of rams during the anoestrous season on the pulsatile secretion of LH in ovariectomized ewes. *J Reprod Fertil*, 67, 47-55, 1983.

12. Martin GB, Milton JT, Davidson RH, Banchero-Hunzicker GE, Lindsay DR, Blache D: Natural methods for increasing reproductive efficiency in small ruminants. *Anim Reprod Sci*, 82-83, 231-245, 2004.

13. Ungerfeld R: Combination of the ram effect with PGF2 estrous synchronization treatments in ewes during the breeding season. *Anim Reprod Sci*, 124, 65-68, 2011.

14. Wright PJ, Geytenbeek PE, Clarke IJ, Hoskinson RM: The efficacy of ram introduction, GnRH administration, and immunisation against androstenedione and oestrone for the induction of oestrus and ovulation in anoestrous post-partum ewes. *Anim Reprod Sci*, 21, 237-247, 1989.

15. Wildeus S: Current concepts in synchronization of estrus: Sheep and goats. *J Anim Sci*, 77, 1-14, 2000.

16. Hawken PAR, Beard AP, Esmaili T, Kadokawa H, Evans ACO, Blache D, Martin GB: The introduction of rams induces an increase in pulsatile LH secretion in cyclic ewes during the breeding season. *Theriogenology*, 68, 56-66, 2007.

17. Martin GB, Oldham CM, Cognie Y, Pearce DT: The physiological responses of anovulatory ewes to the introduction of rams - A review. *Livest Prod Sci*, 15, 219-247, 1986.

18. Ungerfeld R, Rubianes E: Estrous response to ram effect in Corriedale ewes primed with medroxyprogesterone during the breeding season. *Small Ruminant Res*, 32, 89-91, 1999.

19. Martin GB, Kadokawa H: "Clean, green and ethical" animal production. Case study: Reproductive efficiency in small ruminants. *J Reprod Develop*, 52, 145-152, 2006.

20. Smith MF, Swartz, HA, Kiesling DO, Warren JE: Effect of ram exposure and prostaglandin F2 alpha on the reproductive performance of anestrous ewes. *Theriogenology*, 26, 829-835, 1986.

21. Barrett DMW, Bartlewski PM, Cook SJ, Rawlings NC: Ultrasound and endocrine evaluation of the ovarian response to PGF2α given at different stages of the luteal phase in ewes. *Theriogenology*, 58, 1409-1424. 2002.

22. Davies KL, Bartlewski PM, Epp T, Duggavathi R, Barrett DM, Bagu ET, Cook SJ, Rawlings NC: Does injection of prostaglandin F(2alpha) (PGF2alpha) cause ovulation in anestrous Western White Face ewes? *Theriogenology*, 66, 251-259, 2006.

23. Fierro S, Gil J, Vinoles C, Olivera-Muzante J: The use of prostaglandins in controlling estrous cycle of the ewe: A review. *Theriogenology*, 79, 399-408, 2013.

24. Yuksel M, Saat N, Risvanli A: The effect of canine ovariohysterectomy on HSP70 and Anti-HSP70 antibodies. *Kafkas Univ Vet Fak Derg*, 19, 85-88, 2013. DOI: 10.9775/kvfd.2012.7228

25. Knights M, Baptiste QS, Lewis PE: Ability of ram introduction to induce LH secretion, estrus and ovulation in fall-born ewe lambs during anestrus. *Anim Reprod Sci*, 69, 199-209, 2002.

26. Olivera-Muzante J, Fierro S, Lopez V, Gil J: Comparison of prostaglandin- and progesterone-based protocols for timed artificial insemination in sheep. *Theriogenology*, 75, 1232-1238, 2011.

27. Garcia-Palencia P, Sanchez MA, Nieto A, Villar MP, Gonzales M, Veiga-Lopez A, González-Bulnes A, Flores JM: Sex steroid receptor expression in the oviduct and uterus of sheep with estrus syncronized with progesterone or prostaglandin analogues. *Anim Reprod Sci*, 97, 25-35, 2007.

28. Evans ACO, Duffy P, Crosby TF, Hawken PAR, Boland MP, Beard AP: Effect of ram exposure at the end of progestagen treatment on estrus synchronization and fertility during the breeding season in ewes. *Anim Reprod Sci*, 84, 349-358, 2004.

29. Contreras-Solis I, Vasquez B, Diaz T, Letelier C, Lopez-Sebastian A, Gonzalez-Bulnes A: Efficiency of estrous synchronization in tropical sheep by combining short-interval cloprostenol-based protocols and "male effect". *Theriogenology*, 71, 1018-1025, 2009.

30. Pearce DT, Martin GB, Oldham CM: Corpora lutea with a short life-span induced by rams in seasonally anovulatory ewes are prevented by progesterone delaying the preovulatory surge of LH. *J Reprod Fertil*, 75, 79-84, 1985.

31. Oldham CM, Fisher J: Utilizing the 'ram effect'. 33-54. Iowa State University, Iowa State University Extension. Out of Season Breeding Symposium, 1992.

32. McCracken JA, Custer EE, Schreiber DT, Tsang PCW, Keator CS, Arosh JA: A new in vivo model for luteolysis using systemic pulsatile infusions of PGF2α. *Prostag Oth Lipid M*, 97, 90-96, 2012.

33. Kaulfuss KH, Giucci E, Süss R, Wojtowski J: An ultrasonographic method to study reproductive seasonality in ewes isolated from rams. *Reprod Domest Anim,* 41, 416-422, 2006.

34. Maatoug-Ouzini S, Khaldi G, François D, Bodin L: Female response to ram effect in the Barbarine breed: Phenotypic and genetic parameter estimation. *Small Rumin Res*, 113, 376-382, 2013.

35. Khan TH, Beck NF, Mann GE, Khalid M: Effect of post-mating GnRH analogue (buserelin) treatment on PGF2alpha release in ewes and ewe lambs. *Anim Reprod Sci*, 95, 107-115, 2006.

36. Reyna J, Thomson PC, Evans G, Maxwell WM: Synchrony of ovulation and follicular dynamics in merino ewes treated with GnRH in the breeding and non-breeding seasons. *Reprod Domest Anim,* 42, 410-417, 2007.

37. Anel L, Alvarez M, Martinez-Pastor F, Garcia-Macias V, Anel E, de Paz P: Improvement strategies in ovine artificial insemination. *Reprod Domest Anim*, 41, 30-42, 2006.

38. Deligiannis C, Valasi I, Rekkas CA, Goulas P, Theodosiadou E, Lainas T, Amiridis GS: Synchronization of ovulation and fixed time intrauterine insemination in ewes. *Reprod Domest Anim*, 40, 6-10, 2005.

39. Romano JE, Fernandez Abella D, Villegas N: A note on the effect of continuous ram presence on estrus onset, estrus duration and ovulation time in estrus synchronized ewes. *Appl Anim Behav Sci*, 73, 193-198, 2001.