J HELLENIC VET MED SOC 2022, 73 (4): 4757-4762
ΠΕΚΕ 2022, 73 (4): 4757-4762

Research article
Ερευνητικό άρθρο

The effect of reduced dose of $PGF_{2\alpha}$ on certain reproductive parameters in awassi yearlings in anestrus synchronization protocol following the end of the breeding season

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ABSTRACT: The aim of this study was to investigate the effect of the administration of half or full dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Gestavet Prost, d-cloprostenol, HIPRA®) injection in a routine synchronization protocol following the end of the breeding period including intravaginal sponge (medroxyprogesterone acetate; Espanjovet, HIPRA®) and eCG (Oviser 500, HIPRA®) on certain fertility parameters in ewes.Two hundred thirty-eight Awassi yearlings (aged one year old) divided into two groups:116 ewes received a half dose (HSDP; 37.5 µg) of $PGF_{2\alpha}$ and 122 ewes received full dose (FSDP;75 µg).The pregnancy rates were 67.2% and 72.1% for HSDP and FSDP, respectively ($P>0.05$). The lambing rates were 64.7% and 63.9% for HSDP and FSDP, respectively ($P>0.05$).The single lambing rate in HSDP(94.7%) was higher ($P<0.05$) compared to that in FSDP (73.1%). The twin and multiple birth rates of FSDP was higher ($P<0.05$) than HSDP. As a result, it was inferred that aHSDP could be used in Awassi yearlings without experience of lamb care and in a herd in which single lambing was preferable. Moreover, due to the fact that it is more affordable and not detrimentalto fertility, it was concluded that HSDPcan offer an advantage in large flocks and can be used for manipulating fecundity ratesin yearlings.

Key words: Awassi sheep; sponge; reduced $PGF_{2\alpha}$ dose; end of the breeding season; synchronization

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Date of initial submission: 01-07-2021
Date of acceptance: 25-09-2022

INTRODUCTION

Rapid growth of the world population and increased nutrient requirements related to this has forced livestock industry to manage animal reproduction consciously and economically. In today's world, sheep breeding has advantages over cattle breeding due to the grazing ability in non-arable land, having short pregnancy duration, providing animal product such as meat, milk and wool. It is important to maximize output with minimum input to make sheep production efficient (Kaymakcı et al., 2009).

The main objective of sheep production in terms of fertility is to achieve three lambings in two years (Alacam, 2010; Bulbul et al., 2014). While the increase in the birth rates provide more profits and sustainability of the sheep production economically, multiple birth rates can accompany with problem such as higher lamb mortality (Binns et al., 2002). There are many risk factors associated with lamb mortality in the ewes such as lamb birth weight, sex, pneumonia, gastrointestinal diseases, season, trauma, poor mothering pen hygiene, starvation, multiple births, maternal behaviour and care of lambs (Huffman et al., 1985; Stevens et al., 2013; Yapi et al., 1990; Gama et al., 1991; Nash et al., 1996; Binns et al., 2002;Bangar et al., 2016).

One of the reasons of the lamb mortality is multiple births (twin and triple birth) (Huffman et al., 1985; Nash et al., 1996; Binns et al., 2002). Twin and triplet born lambs are more prone to starvation due to the poor dam nutrition and reduced colostrum production by the dam (Refshauge et al., 2016).Also, twin born lambs are smaller and showed weakness in sucking enough amount of the colostrum (Flaiban et al., 2009).It was reported that starvation/exposure accounted for the 45 % of mortality for the multiple born lambs (Scales et al., 1986).Parity arealso related to neonatal lamb mortality (Gama et al., 1991; Dwyer et al., 2005; Mouse-Balabel, 2010). Higher lamb mortality was observed in the primiparous ewes compared to second parity (Gökçe et al., 2013).This can be resulted from lower birth weight of lambs, poor colostrum quality and delayed expressions of maternal behaviour in the primiparous ewes (Dwyer et al., 2003; Levy and Keller, 2008). Refshauge et al. (2016) reported that mismothering and starvation account for 25 % of the neonatal lamb loss in the sheep production. Therefore, manipulating of the litter size can be advantageous in some circumstances in sheep production.

To date, gonadotrophin hormons such as hCG, eCG and GnRH have been used frequently for controlling the litter size in sheep (Cam et al., 2002; Khan et al., 2003; Karaca et al., 2009; Quintero-Elisea et al., 2011). The high dose of eCG results in multiple ovulations, whereas low dose eCG or protocols without eCG causes lower ovulation rates, consequently higher single birth rates (Mutiga and Mukasa-Mugerva, 1992; Quintero-Elisea et al., 2011; Nafaji et al., 2014). It was stated that post-mating hCG and GnRH treatment on day 12 increased the twin pregnancy rates without effecting the single pregnancy rates in sheep (Cam et al., 2002; Cam and Kuran, 2004).Although data on effect of gonadotropins on the litter size in sheep are abundant (Cam et al., 2002; Khan et al., 2003; Karaca et al., 2009; Quintero-Elisea et al., 2011), no evidence was found regarding the effect of $PGF_{2\alpha}$ on litter size and studies regarding clinical implication of reduced dose of $PGF_{2\alpha}$in the sheep reproduction are none.

Based on all this information, the main goal of current study was to reduce the rate of the twin or multiple birth rate in yearling sheep without lamb care experience or in herds in which multiple births are not generally preferred. It was hypothesised that this could be achieved by reducing the dose of $PGF_{2\alpha}$ from a full dose (75 µg) to a half dose (37.5 µg) in the routine synchronization protocol.

MATERIALS AND METHODS

The study was approved by the Office of Animal Ethics Committee of Hatay Mustafa Kemal University (no. 2017/4-4).

Animals

Two hundred thirty eight non-pregnant 45-50 kg weighted Awassi yearlings were chosen from a flock, after the end of breeding period (natural service from August to November) and following pregnancy diagnosis via ultrasonography (USG) (Pie Medical, Falco, Netherlands) 45 days after the removal of the rams. The study was carried out on 22 December 2017 following the end of the breeding period (Akcapınar, 1994).Body condition score was made according to Kenyon et al. (2014). The ewes had an average body condition score of 3and fed with total mixed ration (TMR). Before ram introduction, flushing was done for 30 days with concentrated feed given as 530 gram/animal. Ingredient of TMR were containing bran (10%), maize (52.3%), barley (23%), yeast (1.3%), dicalcium phosphate DCP (0.5%), cottonseed oil meal (1.4%), sunflower seed meal (9%), selenium and zinc (1.5%).As roughage, 1050 gram corn silage, 250 gram straw and 450gram alfalfa were given.

Identifying the number of animals

The number of animals required was determined by taking into consideration previous studies and by sample measurement according to the study hypothesis. Sample measurementsare given below in Table 1, according to confidence level, power of study and rates.

Hormones used in synchronization and determination of the dosage of the prostaglandin $F_{2\alpha}$

Progesterone (Medroxyprogesterone acetate-MPA) containing sponge (60 mg MPA, Espanjovet, HIPRA®, Spain), eCG (500 IU, Oviser, HIPRA®, Spain) and d-cloprostenol (75 µg, GestavetProst, HIPRA®, Spain) were used.Protocols used in the present study are given in Table 2.

Alacam (2007) reported that the dose of $PGF_{2\alpha}$ used in sheep can be half of that used in cow for luteolysis. Thus, a full dose of $PGF_{2\alpha}$ for sheep (FSDP) was defined as 75µg d-cloprostenol by taking into account short product characteristics of Gestavet Prost (d-cloprostenol, 75 µg, HİPRA®) and the dose used for cows for luteolysis (150 µg d-cloprostenol was enough for cows) and 37.5µg d-cloprostenol defined as half sheep dose (HSDP).

Hormonal applications

Both groups were housed together during natural breeding. On 22 December 2017, breeding was started approximately 12 hours after the sponge removal. Ram:ewe ratio was 1:6 and forty rams were held in the flock for 30 days to cover two consecutive estrus cycles.Oestrous behaviours and breedings were not recorded. Pregnancy diagnosis was done via USG with a convex ultrasound transducer (Pie Medical Falco, 2- 5 MHz Convex Probe) at the 45th days after ram's removal (Padilla-Rivas et al., 2005; Suguna et al., 2008). Pregnancy staging was made by determining the ossification in the head, ribs and vertebrae (Ali and Hayder, 2007).Pregnancy records, lamb mortality rate at birth and litter size were recorded.

Fertility parameters

Fertility parameters of animals in the groups were measured with the formulas below.

Pregnancy rate (%) = (Number of pregnant ewes/ Total number of ewes) x 100

Lambing rate(%)= (Number of eweslambing/ Number of pregnant ewes)x100

Single birth rate (%) = (Number of ewes delivered single lamb / Number of ewes delivered) x 100

Twin birth rate (%) = (Number of ewes delivered twins/Number of ewes delivered) x 100

Multiple birth rate (%) =(Number of ewes delivered multiple lambs / Number of ewes delivered) x100

Fecundity rate (%) = (Number of lambs born / Number of ewes delivered) x 100

Lamb mortality rate at birth (%) = (Number of lambs died / Number of lambs born) x 100

Statistical analysis:A comparison of analysed fertility parameters of sheep in groups was made with the SPSS® 22.0 (IBM Corporation, New York NY, USA) program with chi-square (X^2) analysis test.

Table 1. Number of animals in study groups

Rate 1	Rate 2	Confidence level	Power	Sample Size (per group)
0.67	0.53	0.90	0.8	124
0.67	0.52	0.90	0.8	109

Table 2. Protocols with half (HSDP) or full (FSDP) dose of $PGF_{2\alpha}$

Groups	Protocol	Number of animals
HSDP Group	MPA sponges for 7 days + 500 IU eCG and 37.5 µg d-cloprostenol i.m. at sponge removal + natural service by ram introduction on 12 h after sponge removal	116
FSDP Group	MPAsponges for 7 days + 500 IU eCG and 75 µg d-cloprostenol i.m. at sponge removal + natural service by ram introduction on 12 h after sponge removal	122

Table 3. Fertility parameters of ewes received half (HSDP) or full (FSDP) dose of $PGF_{2\alpha}$ during a 7 days synchronization protocol with MAP plus eCG (%; n)

Fertility Parameters% (n)	**HSDP (n=116)**	**FSDP (n=122)**	**X^2**	**P**
Pregnancy rate	67.2% (78)	72.1% (88)	0.674	.412
Lambing rate	64.7% (75)	63.9% (78)	0.013	.908
Single birth rate	94.7% (71)	73.1% (57)	13.037	.000
Twin birth rate	5.3% (4)	15.4% (12)	4.125	.042
Multiple birth rate	5.3% (4)	16.7% (13)	4.973	.026
Fecundity rate	1.05%	1.08%	0.306	.580
Lamb mortality rate at birth	3.8% (3)	9.5% (8)	2.121	.145

X^2: Pearson chi-square value

RESULTS

The fertility parameters measured at the present study in HSDP and FSDP groups are given in Table 3. All pregnant ewes had ossification of head, ribs and vertebrae. Therefore, it was determined that all ewes were conceived in the first estrus after sponge withdrawal. Significant differences were noticed between groups in single lambing rate, twin and multiple birth rates. The percentage of sheep giving single birth in HSDP was higher in comparison to FSDP, whereas twin and triplet births were higher in FSDP group than in HSDP.

DISCUSSION

The aim of this study was to lower the twin and multiple birth rate in yearling sheep without lamb care experience or in herds in which multiple births are not generally preferred.It was hypothesised that this could be achieved by reducing the dose of $PGF_{2\alpha}$from a full dose (75 µg) to a half dose (37.5 µg) in the routine synchronization protocol.Due to the farm and management conditions, natural breeding was performed and oestrous behaviours and breeding date were not recorded. Ultrasonographic examinations were used only for detecting pregnancy. The litter size was identified by considering lambs born per sheep. The results of this study have indicated that reducing HSDPin the routine synchronization protocol ensured similar pregnancy and lambing rates.These results have illustrated that HSDP is as satifying as FSDP in terms of fertility, showing that reducing the dose of $PGF_{2\alpha}$is a cost-effective method regarding the sheep farm management.In the current study, pregnancy rates in the treatment groups were found to be higher than those of other studies (Ataman et al., 2009; Altıncekic and Koyuncu, 2017; Altınçekic et al., 2018; Tajaddodchelik et al., 2020), probably because of different types and dose of gonadotrophins, breed differences, nutrition, duration of the treatment and age of the animals.

The most significant finding of this study was the reduced twin and multiple birth rate and the increased single birth rate in the HSDP in comparison to FSDP. By reducing the dose of $PGF_{2\alpha}$, a lower litter size can be yielded in yearling lambs and sheep herds in which no twinning was preferred. The lower litter size of HSDP might be a result of partial or inadequate luteolysis.Studies regarding effect of different doses of $PGF_{2\alpha}$on luteolysis are available in the literature (Herrera et al., 1990; Juengel et al., 2000; Cerón et al., 2001;Granados-Villarreal et al., 2017). Therefore, it was observed that reduced dose of $PGF_{2\alpha}$ generally led to partial luteolysis and consequently higher levels of progesterone than control group (Juengel et al., 2000;Granados-Villarrealet al., 2017).Progesterone concentration resulting from partial luteolysis affects the oocyte quality negatively, consequently leading to persistent dominant follicles and affects the LH levels (Lonergan et al., 2011).In the case of subluteal or low progesterone level (1-2 ng/ml), frequency of LH pulses increases (Flynn et al., 2000). However, the increase in the LH pulse frequencyis not sufficient for final maturation of dominant follicles and ovulation, leading to persistent dominant follicles (Flynn et al., 2000; Lonergan et al., 2011).Persistent follicles lead to low fertility due to early meiosis by the oocyte reaching metaphase II prior to ovulation. Capacity of these oocytes to fertilise are low. Even if fertilised, they have delayed development causing the increased rates of the embryonic loss at 2-16-cell-stage (Ahmad et al., 1995; Johnson et al., 1996).Vinoles et al. (2001) reported that ovulation of persistent dominant follicles results in the lower pregnancy rates in the ewes.

In the current study, lower multiple and highersingle birth rate in HSDP compared to FSDP might be resulted from subluteal progesterone concentration, increased LH pulse frequency, the persistent follicles containing aged oocytes and increased embryonic loss.The higher single lamb rate can be quite advantageous in terms of lamb mortality.Therefore, this study has indicated that the reduced dose of the $PGF_{2\alpha}$ might be used to decrease the litter size in yearling Awassi ewes, thereby preventing lamb mortality due to litter size and inadequate maternal care of lambs. This treatment could be used in large flocks as there is no difference between HSDP and FSDP groups in terms of fertility parameters and total number of lambs. It has been demonstrated that in addition to eCG, $PGF_{2\alpha}$ can also be used as an alternative tool to control the fecundity rates in yearlings.

CONCLUSION

In brief, the administration of the half dose of $PGF_{2\alpha}$ to a routine synchronization protocol does not have a negative effect on fertility in Awassi yearlings following the end of the breeding season.Taking in mind the results of this study, it was inferred that a half dose of $PGF_{2\alpha}$ together with eCGin a 7 days sponge protocol could be used as an alternative method in yearling lambs without experience in lamb care or herds in which multiple births are not generally preferred. It was considered that with further studies about this topic, a promising tool to control prolificacy in ewe could be improved.

ACKNOWLEDGEMENT

This thesis was supported by Hatay Mustafa Kemal University Scientific Research Committee with the Project number of 18. YL. 015.This research was carried out in the MEMUTA dairy sheep farm, and we thank the MEMUTA Dairy Sheep Farm for their contribution and support.

CONFLICT OF INTEREST

None declared by the authors.

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