Journal of Cell and Animal Biology Vol. 7(2), pp. 16-20, February 2013
Available online at http://www.academicjournals.org/JCAB
DOI: 10.5897/JCAB2013.0204
ISSN 1996-0867 ©2013 Academic Journals

Full Length Research Paper

Effects of prostaglandin $F_2\alpha$ treatment on semen characteristics of crossbred rams in the non-breeding season

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Accepted 4 February, 2013

The aim of the present study was to evaluate the effects of administration of $PGF_2\alpha$ on semen characteristics in crossbred rams in the non-breeding season. Twenty crossbred rams (Arkhar-Merino × Moghani and Arkhar-Merino × Ghezel) were randomly allocated into two equal groups. The experimental group received 7.5 mg IM of Estroplan (PGF2α analogue) and the control group received 1 ml of water soluble. A total number of eighteen ejaculates per ram were collected by artificial vagina twice a week 30 min following IM administration. Semen ejaculates were evaluated for volume, sperm concentration, total sperm output, mass and individual motility, methylene blue reduction time-test (MBRT), percentage of live sperm and sperm abnormality rates. The results of the current study shows that $PGF_2\alpha$ treatment on crossbreed rams improved most semen characteristics including volume, sperm concentration, total sperm output and MBRT in comparison to control group ($P < 0.01$). However, other semen characteristics show similar values. We concluded that $PGF_2\alpha$ actually improved semen output without any negative effect on sperm qualities.

Key words: Arkhar-Merino rams, non-breeding season, prostaglandin $F_2\alpha$, semen characteristics.

INTRODUCTION

The Arkhar-Merino is a sheep breed which was produced by crossbreeding between wild Arkhar rams with ewes of the Novocaucasian Merino, Précoce and Rambouillet breeds (Ernst and Dmitriev, 2007). The targets of producing crossbred rams in northwest Iran were genetic improvement of wool traits in local breeds. Reproduction is one of the most important factors for the economics of livestock production (Quirino et al., 2004). Factors affecting semen characteristics are important in management practices especially for artificial insemination (AI) in sheep breeding programs. It has been demonstrated that exogenous $PGF_2\alpha$ prolonged elevation of blood plasma luteinizing hormone (LH) and testosterone concentrations in a bull (Titiroongruang et al., 2011). However, previous evidence indicates that $PGF_2\alpha$ acts directly at the brain or pituitary rather than the periphery to facilitate secretion of LH and ACTH in the bull (Haynes et al., 1977). Ram seminal plasma is rich in prostaglandins and this hormone has been shown to be of importance to sperm transport in the ewe's cervical canal (Gustafsson et al., 1977).

Treatment with $PGF_2\alpha$ has been used to expedite mounting behavior, as well as, restores libido in bulls displaying decreased sex drive (Masoumi et al., 2011). Use of $PGF_2\alpha$ prior to semen collection may increase the number of sperm in a collection by enhancing sperm movement from the epididymis to the deferent duct, where they are available for ejaculation (Shankar et al., 1984). Estienne and Harper (2004) revealed that there were no exceptional positive or negative effects of long-term treatment with $PGF_2\alpha$ on semen characteristics and libido in boars. The effect of administration of $PGF_2\alpha$ on semen quality has been studied in stallions (Kreider et al., 1981), purebred rams (Mekonnen et al., 1989), boars (Estienne and Harper, 2004; Estienne et al., 2007) and

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bulls (Masoumi et al., 2011). This study concerns effects of $PGF_2\alpha$ on semen characteristics of Arkhar-Merino crossbred rams reared in northwest Iran in the non-breeding season.

MATERIALS AND METHODS

Location

The study was carried out in the non-breeding season (June/July, 2011) at the agricultural research station, University of Tabriz, East Azerbaijan province at 38° 07'N, 46º 29' E and altitude of 1567 m.

Animals and management

Twenty (20) crossbred rams (ten Arkhar-Merino × Moghani; ten Arkhar-Merino × Ghezel), two to three years of age and with an average live weight of 65 ± 5.3 kg were randomly selected from the breeding flock. All these rams were in good health. The animals were maintained under natural photoperiod, and during the trial (9 weeks), the rams were housed separately from ewes. The animals were kept in open front barns; levels of nutrition remained equal as each ram was fed a daily with *ad libitum* diet according to the National Research Council (NRC) containing a 20% concentrate (75% barley, 25% corn, soya and bran) and 80% alfalfa hay.

Treatment schedule

The animals were trained to serve the artificial vagina (AV). The training period lasted four to six weeks. An ovariectomized ewe was used as a teaser and rams were placed in a pen adjacent to the collection area, so that the semen collection procedure could be visualized by each ram (Evans and Maxwell 1987). Animals were randomly allocated into two equal groups (n=10). In the treatment group, the rams were receiving 7.5 mg IM of cloprostenol sodium, a $PGF_2\alpha$ analogue (Estroplan®, Intervet, B.V., Boxmeer, The Netherlands) twice weekly for two months, but in the control group the rams received 1 ml of water soluble. A total number of eighteen ejaculates per rams were collected by AV twice a week 30 minutes following IM administration.

Semen appraising

Immediately following semen collection, the ejaculates were held in a warm water bath at 37°C until their assessment. Semen samples were evaluated for seven characteristics:

1. Volume was measured directly in milliters and with the exactness of 0.1 ml using a glass graduated tube.
2. Sperm concentration was determined using semen diluted with 3% NaCl; the diluted semen was placed on a hemocytometer with the sperm counted in five squares of one chamber (80 small squares with 0.2 mm volume).
3. Total number of sperm per ejaculate was calculated by multiplying sperm density and ejaculate volume.
4. Sperm motility (mass and individual motility) was identified as the percentage of sperm cells that demonstrated progressive motility, from 0 to 100%, by a qualified and experienced investigator. Semen was placed on a heated (37°C) glass slide and scoring was performed at phase-contrast optics (400×). Each sample was evaluated twice. The mean value was used for data analysis.
5. Metabolic activity of spermatozoa was measured by MBRT method (Methylene blue reduction time-test) based on color change from blue to colorless at 37°C. In a thin and transparent tube (1 mm diameter), 0.2 ml semen was added to 0.2 ml of methylene blue and time for color change was recorded.
6. The proportions of live and dead spermatozoa were determined using the nigrosin-eosin staining technique by counting at least 200 spermatozoa under an oil immersion objective (× 1000) in a random field.
7. For abnormal sperm assessment, at least three drops of each sample were added to Eppendorf tubes containing 1 ml of Hancock solution (62.5 ml formalin (37%), 150 ml sodium saline solution, 150 ml buffer solution and 500 ml of double-distilled water) (Schafer and Holzmann, 2000). One drop of this mixture was put on a slide and covered with a cover slip. The percentage of spermatozoa abnormality was determined by counting a total of 200 spermatozoa under phase contrast microscopy (magnification × 1000, using immersion oil). All examinations were performed by the same operator.

Statistical analysis

In a completely randomized design experiment, semen characteristics were analyzed utilizing Proc MIXED procedure of SAS (version 9.1). For volume, Sperm concentration, abnormality and MBRT traits, the outlier data was deleted. The effects of breed and administration of $PGF_2\alpha$ were considered as fixed factors and the effects of ram were considered as a random factor. Spearman correlation tests were used to evaluate the relationship among semen characteristics. Statistical significance was defined as $P < 0.01$.

RESULTS

All of the sperm characteristics in the $PGF_2\alpha$ treated and control groups are set out in Table 1. Treatment with $PGF_2\alpha$ improved some semen characteristics by increasing volume (1.04 vs. 0.82 ml), sperm concentration (3.74 vs. 3.58 $\times 10^9$ sperm/ejaculate), total sperm output (3.62 vs. 3.12 $\times 10^9$ sperm/ml) and MBRT (117 vs. 114 Sec) in the $PGF_2\alpha$ treated rams compared with the control group, respectively ($P < 0.01$). Other seminal variables including mass and individual motility, percentage of live sperm and sperm abnormality rates were not affected by treatment ($P > 0.01$). The Spearman correlation tests between various semen characteristics are summarized in Table 2. Mean volume was correlated significantly with semen concentration and mass motility ($r = 0.55$ and $r = -0.29$; $P < 0.001$), respectively. The correlation coefficient between semen volume and total sperm output was highly significant ($r = -0.31$; $P < 0.001$). Also, total sperm output was significantly correlated with mass motility ($r = 0.28$; $P < 0.001$) in Arkhar-Merino crossbred rams in non-breeding season.

DISCUSSION

Regarding the reproductive performance of Arkhar-Merino crossbred rams and ewes under normal environmental conditions in Iran, little is known. The present study shows that Arkhar-Merino crossbred rams have continuous and acceptable spermatogenic activity

Table 1. Semen characteristics in Arkhar-Merino crossbred rams treated or non treated (control) with 7.5 mg of $PGF_2\alpha$ analogue (Estroplan) in the non-breeding season.

Parameter	$PGF_2\alpha$ nalogue (n=10)	Control (n=10)	P value
	Mean ± SE	Mean ± SE	
SV	1.04 ± 0.22	0.82 ± 0.38	*P* < 0.001
SC	3.47 ± 1.1	3.58 ± 1.3	*P* < 0.01
TS	3.62 ± 1.66	3.12 ± 1.57	*P* < 0.001
IM	67.8 ± 11.3	65.1 ± 10.6	*NS*
MM	4.17 ± 0.51	4.01 ± 0.61	*NS*
LS	70 ± 9.77	68 ± 10.3	*NS*
AS	12 ± 4.44	13 ± 5.45	*NS*
MBRT	117 ± 34.3	114 ± 34.3	*P* < 0.05

NS , Not significant; SV, semen volume (ml); SC, sperm concentration (×109 sperm/ejaculate); TS, total sperm output (×109 sperm/ml); IM, individual motility (%); MM, mass motility (1-5); LS, live sperm (%); AS, abnormal spermatozoa (%); MBRT, methylene blue reduction time-test (Sec).

Table 2. The correlation levels between semen characteristics in Arkhar-Merino crossbred rams treated or non treated (control) with 7.5 mg of $PGF_2\alpha$ analogue (Estroplan) in the non-breeding season.

Parameter	SV	SC	MM	TS	IM	MBRT	LS
AS	0.23*	0.044	-0.84***	-0.11	-0.98***	0.68***	-0.99***
SV		0.55***	-0.29***	-0.31**	-0.25*	0.42***	-0.26**
SC			0.047	0.54***	-0.034	-0.29**	-0.056
MM				0.28**	0.80***	-0.76***	0.80***
TS					0.128	-0.75***	0.122
IM						0.68***	0.98***
MBRT							-0.69***

*Significant at P < 0.05; **Significant at P < 0.01; ***Significant at P < 0.001; coefficients without symbol (*, ** or ***) are not significant. AS, abnormal spermatozoa (%); SV, semen volume (ml); SC, sperm concentration (×109 sperm/ejaculate); MM, mass motility (1-5); TS, total sperm output (×109 sperm/ml); IM, individual motility (%); MBRT, methylene blue reduction time-test (Sec); LS, live sperm (%).

in the non-breeding season. Furthermore, the results of this study indicated that $PGF_2\alpha$ treatment to crossbred rams improved most semen characteristics in the non-breeding season. Apparently, there are differences in degree of response and the effective of prostaglandin among species (stallions; Kreider et al., 1981: purebred rams; Mekonnen et al., 1989: boars; Estienne and Harper 2004: bulls; Masoumi et al., 2011). Azawi et al. (2011) reported that $PGF_2\alpha$ treatment (7.5 mg IM, 3 h prior to semen collection) did not improve most semen characteristics in Awassi rams either in the breeding or non-breeding seasons.

In the present study a significant effect of $PGF_2\alpha$ treatment on ejaculate volume of Arkhar-Merino crossbred rams was observed (*P* < 0.01). This finding is similar to the previous reports showing a significant rise in semen volume in stallions (Kreider et al., 1981), purebred rams (Mekonnen et al., 1989) and bulls (Masoumi et al., 2011), while disagree with the findings of Hafs et al. (1974) and Hashizume and Niwa (1984). They found no effect or improvement of semen characteristics after $PGF_2\alpha$ treatment in rabbits, bulls and boars (Hashizume and Niwa worked with boars). Estienne and Harper (2004) shows that semen characteristics for boars, including semen volume, collected weekly (wk 0 to 16) or daily (four consecutive days) were not affected by treatment. Increased sperm concentration in treated rams agreed with the results obtained by previous authors in boars (Hashizume and Niwa 1984) and Awassi rams (Azawi et al., 2011), while contrary to the findings of Kozink et al. (2002) in boars. Semen volume and sperm concentration of Arkhar-Merino crossbred rams is affected by $PGF_2\alpha$ treatment in the non-breeding season and are important variables in use of AI in sheep breeding programs. Differences in the effectiveness of prostaglandin therapy on semen characteristics reported

by others (stallions; Kreider et al., 1981: purebred rams; Mekonnen et al., 1989: boars; Estienne and Harper 2004: bulls; Masoumi et al., 2011) could be related to the initial libido, genetics, species, age of the animals and to different $PGF_2\alpha$ analogues or their dosages.

The mechanism of increasing ejaculate volume and concentration in response to $PGF_2\alpha$ is not fully understood. It is thought that $PGF_2\alpha$ acts directly on the contractile tissues of the testicular coats and epididymis causing an increased rate of sperm passage from the epididymis to the deferent ducts (Mekonnen et al., 1989). It has been established that smooth muscle surrounding the epididymis contracts in response to $PGF_2\alpha$ in rats (Hib and Oscar 1978). Prostaglandin receptors in the epididymis are most plentiful in the distal segments (Bartke and Koerner 1974), making these areas more sensitive to the changes in $PGF_2\alpha$ concentration. It seems that endogenous prostaglandins affect the caudal epididymis more than other segments of the epididymis. Caudal epididymis acts as a site of storage for mature spermatozoa. When the caudal epididymis contracts in response to $PGF_2\alpha$, mature spermatozoa are moved into the deferent duct where they are available for ejaculation. In the present study the increase in sperm concentration was not accompanied by increase in abnormalities, showing that the action of prostaglandin may be related to the rescue of available mature sperm. In anesthetized rabbits Hafs et al. (1974) demonstrated a significant increase in the movement of sperm from the epididymis to the deferent duct in response to exogenous $PGF_2\alpha$. The speed of appearance of the increased semen output in response to the administration of PGF2α indicates that perhaps the $PGF_2\alpha$ altered the tonicity of the musculature of the excurrent ducts of the reproductive tract of the ram.

The use of $PGF_2\alpha$ treatment in conjunction with semen collection is shown to increase the amount of spermatozoa/ejaculate in the ejaculate of the Arkhar-Merino crossbred rams' semen. Results obtained are in line with previous reports in Holstein bulls (Masoumi et al., 2011). The result of the present study reveals that $PGF_2\alpha$ could be used to enhance spermatozoa numbers in the ejaculate of Arkhar-Merino crossbred ram semen in the non-breeding season. In the current study results considering the percentage of live sperm and sperm abnormalities rates were similar in the $PGF_2\alpha$-treated and control groups, which is similar to the findings of Marshall and Hafs (1976) in the bulls and Azawi et al. (2011) in Awassi rams during breeding and non-breeding seasons. Among type of the spermatozoa abnormality, mostly were observed tail abnormalities.

The mechanism through which $PGF_2\alpha$ affects libido and semen output in the male has not been elucidated. Titiroongruang et al. (2011) reveals that treatment with PGF2α analogue (cloprostenol, 2 ml i.m. 250 µg/ml) in Holstein-Friesian bulls improved the total number of sperm per ejaculate, increased semen output and slightly increased serum testosterone levels in some of the bulls without any negative effect on the sperm qualities. These finding was also shown in the work of Masoumi et al. (2011) injection of PGF2α improved libido, semen quality, and plasma testosterone concentration in low libido Holstein bulls. So, it seems that serum concentration of testosterone is affected by prostaglandin injections. Azawi et al. (2011) reported that the increase in testosterone serum following $PGF_2\alpha$ treatment was thought to be due to direct stimulation of testicular steroidogenesis. Prostaglandin $F_2\alpha$ stimulates cyclic AMP production in the testicle; cyclic AMP then stimulates testosterone synthesis (Reichard et al., 1978). Haynes et al. (1977) concluded that the major effect of systemically administered PGF2a in elevating blood plasma testosterone and glucocorticoid is through LH and presumably adrenocorticotropin release from the pituitary and not directly on the adrenal or testis. This is taken to indicate that elevated plasma steroid concentrations after systemic PGF2a resulted in part, from increased pituitary tropic hormone release

Methylene blue is redox dye that changes color upon reduction by adding of hydrogen. Thus respiration rate of spermatozoa at the dense semen lead to rapid reduction of methylene blue. Thus these samples will become acidic rapidly and long-term storing of these samples is not reliable (Salisbury et al., 1978). Among quantitative traits, highly significant correlations of MBRT with semen volume and sperm concentration were observed (Table 2). Furthermore, a significant correlation was found between total sperm output and motility index, which was in agreement with Kafi et al. (2004) in Persian Karakul rams.

Conclusion

Administration of exogenous $PGF_2\alpha$ significantly improved the semen characteristics including volume, sperm concentration, total number of spermatozoa per ejaculate and methylene blue reduction time-test in Arkhar-Merino crossbred rams in the non-breeding season.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. M. Kafi, Dr. A. Zare Shahneh and Dr. R. Masoumi for their help and collaboration in this work.

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