IJAS Iranian Journal of Applied Animal Science

Relationship of Lipid Factors in Blood Serum and Seminal Plasma of Afshari Rams

Research Article

A. Pirestani[123*] and E. Ziya Motalebipour[234]

[1] Department of Animal Science, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[2] Transgenesis Center of Excellence, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[3] Medicinal Plants Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[4] Department of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

Received on: 7 Feb 2021
Revised on: 25 Mar 2022
Accepted on: 14 Apr 2022
Online Published on: Sep 2022

*Correspondence E-mail: a.pirestani@khuisf.ac

Online version is available on: www.ijas.ir

ABSTRACT

The aim of this study was to investigate the relationship of lipid factors of seminal plasma and blood serum in Afshari rams. Four Afshari rams were selected with a mean weight of 50 ± 5 kg and the mean age of 2.5 years. At the same time, blood and semen samples were collected to evaluate total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), while beta-hydroxybutyric acid (BHBA) was measured only in blood samples. After adding semen to the extender, sperm motility, morphology and viability were evaluated by the CASA software, Papanicolaou and Eosin-Nigrosin staining, respectively. The sperm parameters of seasonal and non-seasonal breeding were assessed in pre-freezing and post-thawing. The results of this study showed that cholesterol and HDL levels of ram were significantly higher in seasonal breeding than non-seasonal breeding in both blood and semen samples ($P<0.05$). In non-seasonal breeding, LDL and triglyceride levels of blood and semen samples were significantly increased respectively ($P<0.05$). In seasonal breeding, the sperm parameters such as motility, morphology, and viability of rams were significantly higher in pre-freezing. In non-seasonal breeding, total motility was higher significant in post-thawing ($P<0.05$). Consequently, in the current study, there was a significant correlation between triglyceride of blood and triglyceride of seminal plasma. Also, there was a significant correlation between blood LDL and seminal LDL/HDL ratios for non-seasonal records. Blood LDL/HDL was also significantly correlated with the seminal cholesterol/HDL, which could improve the sperm quality parameters of Afshari ram in the season and non-seasonal breeding.

KEY WORDS Afshari ram, blood, lipid, seminal plasma, sperm.

INTRODUCTION

The ejaculated sperm has a high cholesterol to phospholipid ratio. When the sperm is placed under laboratory conditions, its cholesterol content decreases. During sperm motility, cholesterol moves from the plasma membrane to the proteins that it receives. Also, phospholipids are removed from the sperm plasma membrane, which causes to occur acrosomal reactions (Frenkel *et al*. 1974). During the acrosome reaction, the ratio of cholesterol to phospholipid is reduced. The rate of sperm capacitation is dependent on the rate of cholesterol drainage from the plasma membrane. Sperm capacitation changes included the reorganization of membrane proteins, metabolism of membrane phospholipid, and the reduction of membrane cholesterol levels; changes in sperm motility can increase the kinetics of the sperm to the oviduct for penetrating the zona pellucida. Sperms of some species, such as humans, bulls and stallions have high cholesterol levels and low rates of capacitation. In contrast, ram and pig sperms have low cholesterol

content, which results in a high level of capacitation. Some research has been shown that freezing has an effect on sperm quality, which resulted in a reduction of sperm cholesterol and finally resulted in a decrease in sperm motility and acrosome reaction (Maldjian *et al*. 2005).

It has also been shown that cholesterol plays an important role in the freezing of sperm and extender compounds. On the other hand, semen plasma contains cholesterol, triglycerides and other compounds that is needed for sperm energy. Therefore, this study was conducted to evaluate the level of cholesterol in blood serum and semen on motility, viability and morphology parameters in seasonal and non-seasonal breeding of Afshari rams in pre-freezing and post-thawing and also to investigate the correlation between these parameters.

MATERIALS AND METHODS

All chemical reagents were obtained from Merck (Darmstadt, Germany), unless otherwise noted.

Blood and semen collection, evaluation and sample preparation

Semen samples were collected from 4 mature Afshari rams (weight, 50±5 kg; age, 2.5 years) maintained at the Animal Breeding Center Farm, Isfahan Branch, Islamic Azad University, Iran. The rams were daily fed with 0.91 kg of concentrate while water and good quality hay were supplied *ad libitum.* Semen samples were collected from the rams using the artificial vaginal (*imv*, Frances) two times per each ram and twice a week during the breeding season (autumn to early winter) and non-seasonal (late spring to early summer).

After semen collection, the raw semen samples were immediately transferred to the laboratory, and kept in a water bath at 37 ˚C for further examination. Sperm concentration was measured with a photometer. Semen samples were pooled to eliminate individual differences. Spermatozoa that showed > 70% progressive motility and 2.5 × 10^9 spermatozoa/mL were selected for more experiments (Aitken, 1990). However, at the same time of semen sampling, blood samples were collected from a jugular vein and their serum were separated in a laboratory.

Extender preparation

An extender (10 mL) was prepared in autoclaved double distilled water (prepared in a Transgenesis Center of Excellence, Islamic Azad University of Isfahan) containing 0.244 g Tris, 0.136 g citric acid, 0.082 g D-fructose and 20% v/v egg yolk (Fiser *et al*. 1987; Yaniz *et al*. 2010).

Experimental design

In this study, semen samples were collected in seasonal and non-seasonal breeding and then were added to an extender. Sperm parameters were evaluated for 20 min post incubation at 37 ˚C by the CASA software, Papanicolaou and Eosin and Nigrosin staining as pre-freezing. Afterwards, semen samples were freezed and one week later was thawed and sperm parameters were analyzed again. In a laboratory, total cholesterol, triglyceride, HDL and LDL concentrations were evaluated in the sperm samples with a biochemical kit by auto analyzer Mindray BS 800 at fresh sperm. A 10-cc blood sample was collected and total cholesterol, triglyceride, HDL and LDL concentrations of the blood sample were evaluated with a biochemical kit.

Sperm motility

Sperm motility was evaluated using a computer automated semen analysis (CASA analyzer, video sperm test 2.1), and by an Olympus BX40 microscope under 100 × magnifications on a warm stage with 37 ˚C (Joshi *et al*. 2003; Kumar *et al*. 2007). The sperm motility parameters [fast forward moving sperm (A motility), slow forward moving sperm (B motility), not forward moving sperm (C motility), non-moving sperm (D motility), percentage of progressive motility (PM %) and percentage of total motility (TM %)] were assessed in 10 microscopic fields from each slide which include at least 300 sperm cells.

Sperm morphology

Morphology was evaluated by direct microscopic examination using a Papanicolaou staining technique according to strict criteria. Normal and abnormal sperm cells were counted by the CASA software using 200 sperm cells from each group (WHO, 2010; Figure 1).

Sperm viability

Eosin and Nigrosin (E&N) staining was carried out according to the Björndahl *et al*. (2003) study. Briefly, 1% Eosin and 10% Nigrosin was prepared in distilled water and a 1:2 admixture of semen to the prepared eosin (v/v) was made successively. After 30 seconds, an equal volume of Nigrosin was added to this mixture, then thin smears were prepared and observed by light microscopy at 100 X magnification. A viable sperm remained colorless while a nonviable sperm stained red.

Statistical analysis

Data were analyzed using SPSS (2011). Statistical analysis was carried out using the ANOVA procedure and the mean comparison was conducted by LSD test.

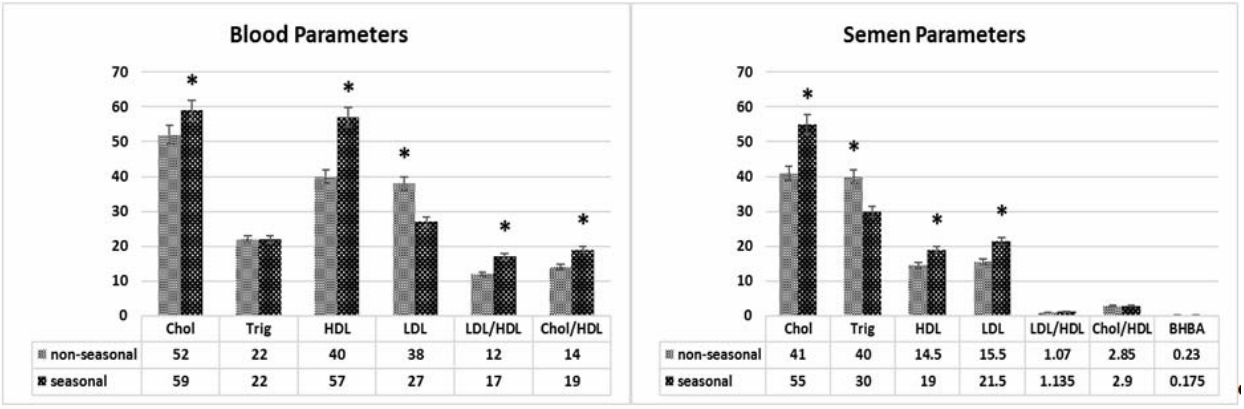

Figure 1 Blood and semen lipid parameters in seasonal/non-seasonal breeding

A P-value < 5% was considered as a statistically significance level. The Pearson correlation coefficients were calculated using Microsoft Excel. Based on the online tool available at the website of Social Science Statistics (https://www.socscistatistics.com/pvalues/pearsondistribution.aspx), only P-values > 0.81 were considered significant at alpha of 0.05 and sample size of 6.

RESULTS AND DISCUSSION

The mean of blood lipid parameters has been shown in Figure 1. The results of this study showed that the total serum cholesterol, HDL and LDL levels in blood of the Afshari rams was high and significantly different in the breeding season compared to the non-breeding season (P<0.05). Triglyceride concentration in the breeding season was in the same level as the non-breeding season. Furthermore, LDL/HDL and cholesterol/HDL parameters were increased in the breeding season (Figure 1).

The mean of semen lipid parameter has been shown in Figure 1. Also, the results showed that there was a significant difference regarding semen cholesterol, HDL and LDL concentrations in semen of the Afshari rams in the breeding and non-breeding seasons; the levels of these parameters were significantly higher in the breeding season compared to the non-breading season. However, the triglyceride concentration in semen of the rams was significantly increased in the non-breeding season (P<0.05) compared to the breeding season. There was no significant difference in LDL/HDL, cholesterol/HDL and beta- hydroxybutyric acid (BHBA) parameters between the breeding and non-breeding seasons (Figure 1). The correlation coefficients were calculated for various parameters between the blood and seminal plasma. Results showed that none of the cholesterol, HDL, and BHBA parameters had significant correlations with themselves and other parameters between blood and seminal plasma (Table 1).

However, there was a significant correlation between triglyceride of blood and triglyceride of seminal plasma. Nonetheless, neither of other parameters were significantly correlated with one another in blood and seminal plasma. Additionally, there was a significant correlation between blood LDL and seminal LDL/HDL ratios for non-seasonal records. Blood LDL/HDL was also significantly correlated with the seminal cholesterol/HDL attribute. Moreover, the blood cholesterol/HDL was also significantly associated with the seminal LDL as well as LDL/HDL ratio.

In the current study, TM and C motility were significantly higher in the seasonal breeding in pre-freezing evaluation compared to non-seasonal breeding (P<0.05). However, progressive motility (PM), A motility and D motility were significantly increased during the non-seasonal breeding in pre-freezing (P<0.05). Moreover, no significant difference was found in B motility between seasonal and non-seasonal breeding in pre-freezing evaluation (Figure 2). According to the post-thawing evaluation, TM and C motility were significantly higher in the non-seasonal breeding compared to those of the seasonal breeding (P<0.05) whereas, A motility and D motility were significantly increased in the seasonal breeding (P<0.05). Furthermore, the PM and B motility were not significantly different during the non-seasonal breeding in post-thawing evaluation. Motility of sperm was significantly increased in the seasonal breeding during pre- and post-freezing evaluations (Figure 2). Sperm morphology data in the seasonal and non-seasonal breeding are summarized in Table 2. During pre-freezing evaluation in the breeding season, the percentage of normal morphological features of the sperm was 87% while abnormal morphological features of the sperm was 13%. In the post-thawing stage, the percentage of normal morphological features of the sperm was 81%, while the percentage of abnormal morphological features of the sperm was 19% in the seasonal breeding.

Table 1 Pearson correlations[1] for different factors between blood and seminal plasma

Semen \ Blood	Cholesterol	Triglyceride	HDL	LDL	LDL/HDL	Cholesterol/ HDL	BHBA
Cholesterol	15.7 & 24.9	54.8	51.4	18.1	-59.7	80.1	-35.3
Triglyceride	80.0	-91.5* & 5.9	-2.2	54.1	76.3	-24.8	39.1
HDL	-28.3	-7.5	-50.1 & 19.0	5.5	-12.2	24.7	-75.0
LDL	5.8	0	5.6	41.0 & 44.7	-41.5	98.7*	3.8
LDL/HDL	-79.5	50.7	0	95.7*	11.7 & -25.7	86.1*	12.5
Cholesterol/HDL	-4.9	6.6	2.7	45.1	82.0*	77.8 & 17.0	-66.0
BHBA	48.9	0	5.6	-11.1	38.0	74.6	NA

[1] The correlation coefficients for seasonal and non-seasonal records are provided in upper and lower triangles, respectively. The correlation coefficients for each parameter between blood and seminal plasma are depicted as the diagonal elements for seasonal and non-seasonal records, respectively.
HDL: high-density lipoprotein; LDL: low-density lipoprotein and BHBA: beta-hydroxybutyric acid.
* (P<0.05).

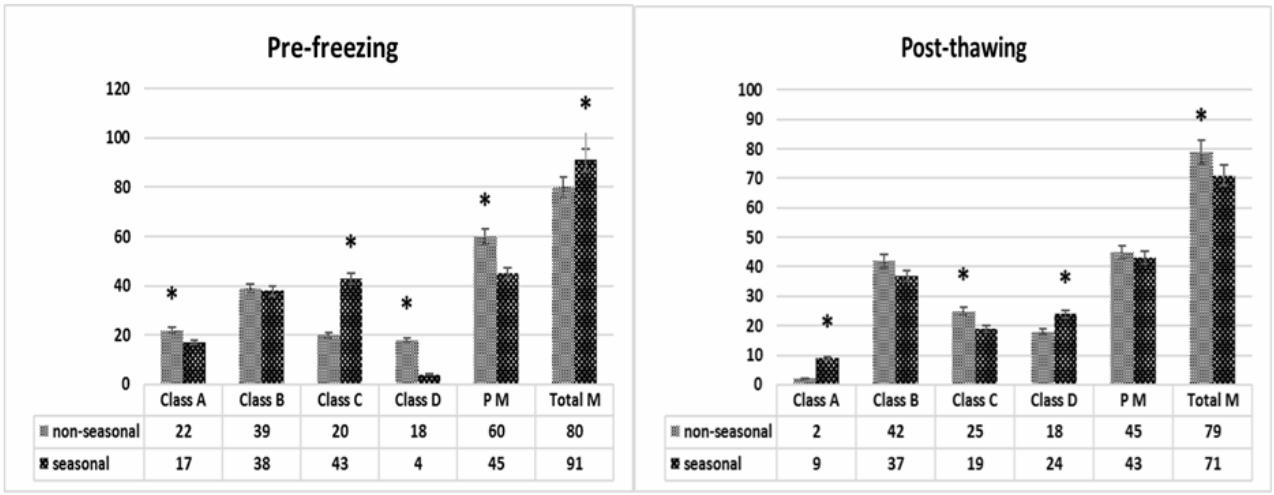

Figure 2 Evaluation of sperm motility in seasonal/non-seasonal breeding at pre-freezing and post-thawing

Table 2 Comparison of ram sperm morphology in two seasonal/non-seasonal breeding and two different times

Item	Morphology (%)	Treatment groups	
		Seasonal	Non-seasonal
Pre-freezing	Normal	87*	55
	Abnormal	13	45
Post-thawing	Normal	81*	52
	Abnormal	19	48

* (P<0.05).

Therefore, a significant difference was observed between normal and abnormal morphological features of the sperm in both pre-freezing and post-thawing stages (P<0.05). During the pre-freezing stage, the percentage of normal morphological features of the sperm was 55% while abnormal morphological features of the sperm was 45% in the non-seasonal breeding.

During the post-thawing stage, the percentage of normal morphological features of the sperm was 52% while abnormal morphological features of the sperm was 48% in the non-seasonal breeding stage; there is no significant difference in this evaluation.

Viability of the sperm during pre-freezing and post-thawing stages have been shown in Table 3.

Viability of the sperm was grouped as two classes: dead and live sperms. During pre-freezing in the breeding season, the percentages of live and dead sperms were 80% and 20%, respectively. The similar evaluation was observed in the post-thawing stage with 75% of live sperm and 25% of dead sperm in the breeding season. In the pre-freezing stage, the percentages of live and dead sperms were 67% and 33%, respectively, in the non-seasonal breeding. Also, during the post-thawing stage, the percentages of live and dead sperms were 60% and 40%, respectively, in the non-seasonal breeding.

A significantly high level of live sperm was detected in the seasonal and non-seasonal breeding in both pre-freezing and post-thawing stages (P<0.05).

Table 3 Comparison of ram sperm viability in two seasonal/non-seasonal breeding and two different times

Item	Viability (%)	Treatment groups	
		Seasonal	Non-seasonal
Pre-freezing	Live	80*	67*
	Dead	20	33
Post-thawing	Live	75*	60*
	Dead	25	40

* (P<0.05).

This study aimed at evaluating the effect of lipids concentrations in blood serum and semen on motility, viability and morphology parameters in seasonal and non-seasonal breeding of Afshari rams during pre-freezing and post-thawing stages. According to the results of the present study, high concentrations of cholesterol and HDL in blood of the rams could be due to dietary concentrate. Therefore, increased levels of dietary concentrate in blood serum can causes an increase in this lipid in semen in seasonal breeding (Herdt, 2000; Beer-Ljubic *et al*. 2009).

In the current study, the concentrations of LDL in blood of the rams and triglyceride in the semen were increased through non-seasonal breeding, which can be due to the composition of the diet and the amount of grains in the diet. The results of the present study were consistent with the results of the Beer-Ljubic *et al*. (2009) study.

The results of this study evinced that TM of the ram sperm was significantly increased in the seasonal breeding during the pre-freezing stage. The high evaluation of TM can be affected by hormonal changes. Furthermore, an increase of lipids in the blood serum and semen before freezing (the pre-freezing process) could be explained by increasing the health of the sperm plasma membrane as well as by increasing the motility of it. Additionally, the progressive motility of sperm in the non-seasonal breeding was higher than that in the seasonal breeding in the pre-freezing process, which can be related to high amounts of triglyceride in semen. Triglyceride concentrations of semen are needed for oxidation and energy supply of sperm. Increasing triglyceride concentrations in semen can be caused by the rise the very low-density lipoprotein (VLDL) receptor expression on the sperm membrane, which followed by increasing of the motility of sperm (Beer-Ljubic *et al*. 2009). In addition, a high LDL concentration in blood serum in non-seasonal breeding has an effect on sperm membrane and eventually can progress TM of sperm (Liu *et al*. 2017).

The results of morphology and viability of sperm in the pre-freezing stage represented a high quality of sperm in the seasonal than non-seasonal breeding. One of the factors affecting sperm quality is an increase in the level of lipid in blood serum and semen, which improves the plasma membrane of the sperm.

As a result of improving the plasma membrane of sperm, the level of viability and improving of sperm morphology will be achieved (Am-In *et al*. 2011).

After the freezing-thawing process, TM of Afshari ram sperm was significantly higher in the non-seasonal breeding compared to the seasonal breeding. A high level of TM can be due to cold shock in the plasma membrane of sperm. During cold shock, from the surface of the sperm membrane, the hyaluronidase and glutamic oxaloacetic transaminase enzymes are released. There is a negative relationship between the release of these enzymes and the fertility sperm after freezing-thawing (Barbas and Mascarenhas, 2009).

It seems that the release of the enzymes is also affected by extender and seminal plasma. Increasing the lipid concentration can reduce the released enzymes from the surface of the sperm membrane (Beer-Ljubic *et al*. 2009; Liu *et al*. 2017).

According to the results of the present study, morphology and viability were improved in the seasonal breeding at the freezing-thawing process. The high amount of LDL, HDL and cholesterol can affect this improvement. Furthermore, increased levels of semen lipids were resulted in the resistance of sperm against heat injuries and improvement of sperm motility after the freezing-thawing process (Am-In *et al*. 2011).

CONCLUSION

In this study, blood lipid levels in the seasonal and non-seasonal breeding are different in the blood and seminal plasma in Afshari rams. High levels of cholesterol and HDL in blood samples can increase the amount of these factors in seminal plasma; which can improve the sperm parameters in seasonal breeding. The levels of triglyceride and LDL were increased in blood and seminal plasma in non-seasonal breeding, which has a beneficial effect on TM and sperm parameters after a freezing-thawing process.

ACKNOWLEDGEMENT

The authors thank all the teams who worked on the experiments and provided results during this study.

REFERENCES

Aitken R.J. (1990). Motility parameters and fertility. Pp. 285-302 in Control of Sperm Motility: Biological and Clinical Aspects. C. Gagnon, Ed. CRC Press, Boca Raton, Florida.

Am-In N., Kirkwood R.N., Techakumphu M. and Tantasuparuk W. (2011). Lipid profiles of sperm and seminal plasma from boars having normal or low sperm motility. *Theriogenology.* **75(5),** 897-903.

Barbas J.P. and Mascarenhas R.D. (2009). Cryopreservation of domestic animal sperm cells. *Cell Tissue Bank.* **10(1),** 49-62.

Beer-Ljubić B., Aladrović J., Marenjak T.S., Laškaj R., Majić-Balić I. and Milinković-Tur S. (2009). Cholesterol concentration in seminal plasma as a predictive tool for quality semen evaluation. *Theriogenology*. **72(8),** 1132-1140.

Björndahl L., Söderlund I. and Kvist U. (2003). Evaluation of the one#step eosin#nigrosin staining technique for human sperm vitality assessment. *Hum. Reprod*. **18(4),** 813-816.

Fiser P.S., Ainsworth L. and Fairfull R.W. (1987). Evaluation of a new diluent and different processing procedures for cryopreservation of ram semen. *Theriogenology*. **28(5),** 599-607.

Frenkel G., Peterson R.N., Davis J.E. and Freund M. (1974). Glycerylphosphorylcholine and carnitine in normal human semen and in postvasectomy semen: Differences in concentrations. *Fertil. Steril*. **25(1),** 84-87.

Herdt T.H. (2000). Variability characteristics and test selection in herd-level nutritional and metabolic profile testing. Veterinary clinics of North America, *Food Anim. Pract.* **16(2),** 387-403.

Joshi A., Naqvi S. M.K., Bag S., Dang A.K., Sharma R.C., Rawat P.S. and Mittal J.P. (2003). Sperm motion characteristics of Garole rams raised for a prolonged period in a semi-arid tropical environment. *Trop. Anim. Health Prod*. **35(3),** 249-257.

Kumar D., Joshi A., Naqvi S.M.K., Kumar S., Mishra A.K., Maurya V.P., Arora A.L., Mittal J.P. and Singh V.K. (2007). Sperm motion characteristics of Garole × Malpura sheep evolved in a semi-arid tropical environment through introgression of FecB gene. *Anim. Reprod. Sci*. **100(1),** 51-60.

Liu C.Y., Chou Y.C., Lin S.H., Wu S.T., Cha T.L., Chen H.I. and Tsao C.W. (2017). Serum lipid profiles are associated with semen quality. *Asian J. Androl*. **19(6),** 633-640.

Maldjian A., Pizzi F., Gliozzi T., Cerolini S., Penny P. and Noble R. (2005). Changes in sperm quality and lipid composition during cryopreservation of boar semen. *Theriogenology*. **63(2),** 411-421.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

World Health Organisation. (1999). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, New York.

Yániz J.L., Marco-Aguado M.A., Mateos J.A. and Santolaria P. (2010). Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 C. *Anim. Reprod. Sci*. **122(1),** 142-149.