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EFFECT OF AGE ON SEMEN PARAMETERS AND SPERM DNA FRAGMENTATION IN IRAQI AWASSI RAMS

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ABSTRACT : The study was conducted on 7 Awassi rams, aged between 8-11 months, found in farm of College for Veterinary Medicine, Fallujah University, Al-Anbar province, the period of experiment (October 2018-March 2019). The animals were fed alfalfa and hay, the water was given adlibtum. Semen was collected at morning via electro-ejaculator, one ejaculation per week with 12 ejaculation per each ram, 6 before maturity (8-11 months age) while 6 after maturity (more than 12 month age), semen evaluation and DNA extraction to detect DNA fragmentation are made to the fresh semen. The result showed that there is significant differences in semen parameters in different ages in which the semen parameters in rams more than 14 months were better than rams less than 1 years old also the younger rams have a high percentages of sperm DNA fragmentation in contract with the older rams.

Key words : Semen, Awassi rams, sperm DNA, effect of age.

INTRODUCTION

The sperm production regarded as an important tool which takes place in the presented applies of reproduction in sheep, with an aiding of artificial insemination, it improve the animal genotypes as well as the conservation of the genetic inheritance, the sperm production analysis is of unlimited significance because its correlated significantly with a sexual activity.

Sperm production and quality increment with age after adolescence (Tabbaa *et al*, 2006; Štolc *et al*, 2009; Focºãneanu *et al*, 2014), then plateaus approximately 3 years of age (Hassan *et al*, 2009). Several studies have shown a significant decline in ejaculate quality in aged rams (Chella *et al*, 2017). This decline in sperm quality with age has been attributed to the beginningof testicular degenerative changes.

Ram's age considered as a major factor that causing differences in Scrotal circumference and/or characteristics of semen (Tabbaa *et al*, 2006; Toe *et al*, 1994) with Scrotal circumference are closely correlated to total sperm production (Ahmad and Noakes, 1995). Although, the information which available on the characteristics of semen for several breeds of sheep are very little is known regarding to seasonal variation in characteristics of semen of Awassi sheep for different ages (Gundogan and Serteser, 2005).

The spermatozoa were considered to be more sensitive to the oxidative damage due to containing a higher levels of poly-unsaturated fatty acids (PUFAs) founding in plasma membrane (Vernet *et al*, 2011). The yearling ram have a high level of (PUFAs) that lead to increasing in oxidative stress and finally sperm DNA fragmentation.

DNA fragmentation is a biochemical distinguishable aspect of apoptosis. The Apoptosis was arbitrate by the proteolytic enzymes, which is called caspases, which trigger the cell death by splitting the specific proteins in the nucleus and cytoplasm (Bruce *et al*, 2002). It is also known as Caspase Activated Nuclease, DNA Fragmentation (Yuste *et al*, 2014). So the electrophoresis is a method for separation of the macromolecules like DNA, RNA, the proteins and their fragments based on two factors, the size and the charge (Kryndushkin *et al*, 2003).

In view of this and since little information are available regarding this field in ram semen and their sperm DNA

fragmentation. The aim of the current study was to examine the effects of age on sperm parameters and semen DNA fragmentation in Iraqi awassi rams.

MATERIALS AND METHODS

The present study was conducted on 7 Awassi rams, aged between 8-11 months, which found in the Farm of Veterinary Medicine College, Fallujah University, Al-Anbar province during a period (October 2018-March 2019). The animals were fed alfalfa and hay the water was given adlibtum.

Semen evaluation

Semen samples were collected from each ram with electro ejaculator (Electro jac5/Aneogen company, U.S.A.). Semen samples were physically evaluated and genetically for genomic DNA fragmentation using electrophoresis. One ml of semen sample was taken for determination of semen parameters. Volume of the semen was directly measured by reading of graduated marks of collecting tubes according to Mortimer (1994). pH of semen was determined by papers trips by dipping the indicator pH paper into the tube which contain the samples and observation changing in the color of paper and then compared with a table that has a pH for each color (Salisbury *et al*, 1978).

Mass motility has been done by putting a drop of fresh undiluted semen on a warm slide at 36^0C and examined under light microscope supplied with heat stage at 100× magnification. Estimate the swirl grade according to Chenoweth (2002). The grades include Rapid swirl (very good), slower swirl (good), general oscillation (fair), sporadic oscillation (poor). Individual motility was measured by taking one drop of fresh semen plus one drop of sodium citrate and measured the percent of individual motility. Two smears of semen which stained with (E/N) eosin-nigrosine were prepared (Blom, 1950) and used to determine the percent of live/dead and morphological abnormal spermatozoa (primary and secondary) according to Bielanski *et al* (1982). Sperm concentration was calculated with hemocytometer chamber (Salisbury *et al*, 1978).

Genetic examination

After semen collection semen samples were transported to the lab. ineppindorf tubes and using cooling container and then examined for DNA fragmentation by a special kit (EXTRACTME)®, after DNA extraction using of electrophoresis to get the results.

Agarose gel is prepearing as described by (Sambrook and Russel, 2001). Fifty ml of TBE buffer 1X was taken. The agarose powder was added to the buffer in a suitable concentration. The solution was heated until boiling by using microwave till all gel particles were dissolved. Then the solution was left untill cool at 50-60°C, then 1 µl of ethidium bromide 10 mg/ml added to the agarose solution and the agarose was stirred in order to be mixed and must avoid making bubbles and then used in electrophoresis.

Electrophoresis

Electrophoresis, which was used foranalyses the extracted DNA by agarose gel electrophoresis as reported by Brody and Kern (2004).

1. Agarose gel 2% was prepared in 1X TBE buffer and heated by hot magnetic stirrer until all crystals were disappeared in agarose.
2. After cooling 3µl of Ethidium bromide per 100 ml gel solution (special nucleic acid stain) were added.
3. The gel was emptied in a tray and fixed the comb at the right position and left until solidifying, then the comb removed carefully.
4. Ten µL of exracted DNA were dripped into each comb well.
5. Then extracted DNA were transferred into electrophoresis machine, which contained the same 1X TBE buffer that used in preparation of agarose gel.
6. Then an electric current was set up at 70 Volt and 100 AM for 1hour.
7. Finally extracted DNA bands were visualized using a UV transilluminator and photographed by using digital camera.

Statistical analysis

Data were analyzed by using t-test, SPSS program (Version 20.0) (Daniel, 1991).

RESULTS AND DISCUSSION

It has been observed that there are different definition of puberty constitute the presence of well-developed genital system, libido and minimum characteristic of semen ejaculate (Abraham *et al*, 2016). It has been reported that the male for the first time can mate the female and give rise to the pregnancy. It's found that the differences observed in the onset of puberty are might be attributed to many factors including breed, climate, nutrition, management, the methods of measuring puberty and the semen collection methods (Elhamamli *et al*, 2013).

The parameters of semen is shown in Table 1. There was a significant differences ($P<0.05$) in semen values before and after maturity. Similar observation have been made by Ghorbankhani *et al* (2015). The results are in

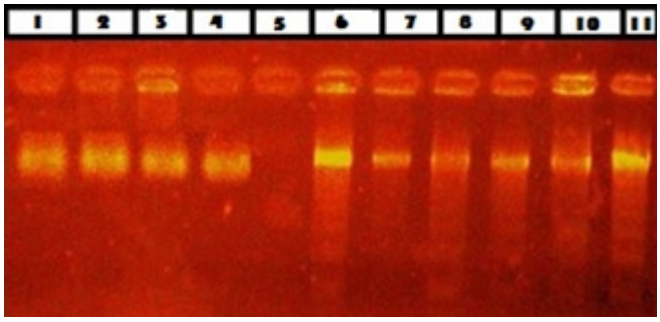

Fig. 1 : Normal DNA and fragmented DNA.

Table 1 : The semen parameters in yearling and mature awassi rams.

Parameters	Yearling rams	Mature rams
Volume	0.91 ±0.042B	1.16±0.053A
pH	6.87±0.019B	7.02±0.021A
Concentration × 10^7	194.02±11.42B	216.97±8.01A
Abnormalities %	20.90±0.91A	15.40±1.0B
Dead sperms %	40.81±1.57A	22.47±0.86B
Mass motility %	36.57±0.92B	72.02±0.95A
Individual motility %	60.71±1.18B	75.47±1.11A

• The different capital letters refer to significant differences between different groups at (P<0.05).

agreement with results of Boussena *et al* (2016).

The average volume of rams sperm per ejaculate in awassi breed was 0.91-1.16 ml which also supports the results of Hafez (2000).

The current results showed that values of sperm concentration in a yearling rams were significantly lower than those in mature one. The same results was getter by Aissaoui *et al* (2004), Ghozlane *et al* (2005). This distinction because of a differences in the age which reported by numerous studies that highlighted a solid positive association between the concentration of semen and the age of animals (Rege *et al*, 2000; Okukpe *et al*, 2001; Salhab *et al*, 2003; Recabarren *et al* (2008), Hassan *et al* (2009).

An average value of the mass motility is very acceptable (Table 1) and considered very good parameterin older rams according to Baril *et al* (1993). The sperm in this case is acceptable and appropriate for an insemination. The present study showed that mean of rams sperm mass motility was higher than that suggested by Ghozlane *et al* (2005).

The pH result also in agreement with Salhab *et al* (2001). The vaiablity result in agreement with Hassan *et al* (2009). While the abnormalities result in agreement with Azubuike *et al* (2017).

The result showed that the maturity of lamb ram reaches at 48-56 weeks, which consider the perfect age for best result of semen quality in awaasirams. These results are disagreed with with the result of El-Ashry *et al* (2000), who found that lambs reaches to maturity at the age ranged (36-40) weeks. This might be due to this study was done duringn on-breeding season.

Genomic DNA extracted running in the electrophoresis the samples were appeared the normal and fragmentation extracted samples.

In Fig. 1, the numbers (1-4) denotes to normal DNA (older rams), while the numbers (6-11) denotes to the fragmented DNA (yearling rams). Spermatozoa are consider sensitive to ROS or an oxidative damage because of the containing a higher content of PUFAs in a plasma membrane (Vernet *et al*, 2011). Also, Kelso *et al* (1997) reported that the PUFA playing a great role in the lipid metabolism of sperms, the motility and even ability to fuse with an oocyte. A decreasing in the percentages of PUFA in lipids of sperm was convoyed by a decrease in the sperm number and motility in ejaculates of aged animals *i.e.* the yearling ram have a high level of(PUFAs) that lead to increasing in oxidative stress and finally sperm DNA fragmentation which in agreement with the results of this study.

Although, PUFAs are also associated with increased (ROS), which might have a detrimental effect on testes and reduce the semen quality (Van Tran *et al*, 2016).

Ram spermatozoa can produce a higher levels of H_2O_2, particularly due to contain a higher amounts of PUFAs and a low amounts of cholesterol/phospholipids in the plasma membrane as compared with other species. These percentages are accountable for an increased susceptibility to oxidative damage in the ROS presence, and consequent damage of membrane and acrosome integrity (Alvarez *et al*, 1992). As the stress factor participate for liberate the radical oxygen species reason for damage of mitochondrial DNA with the enzyme of cells as suggested by Matsuoka *et al* (2000).

CONCLUSION

The effect of ages were demonstrated in rams to be a factor that effects on semen parameters. A quantitative sperm production including all parameters, which higher in adult age than in younger ages. Sperm DNA fragmentation is higher in young than in adults rams because of high levels of polyunsaturated/saturated fatty acids presented in semen.

REFERENCES

Abraham M, Puhakka J, Ruete A, Al-Essawe E, de Verdier K, Morrell J and Båge R (2015) Testicular length as an indicator of the onset of sperm production in alpacas under Swedish conditions. *Acta Veterinaria Scandinavica* **58**(1).

Agarwal A and Allamaneni S S (2004) The effect of sperm DNA

damage on assisted reproduction outcomes. A review. *Minerva Ginecol*. **56**(4), 235-245.

Ahmad N and Noakes D E (1995) Seasonal variations in testis size, libido and plasma testosterone concentrations in British goats. *J Anim Sci*. **61**, 553-559.

Aissaoui C, Chibani J and Bouzebda Z (2004) Etudes des variations de la production spermatique du bélier de race OuledDjellalsoumis à un régime pauvre. *Renc.Rech. Ruminants* **14**, 380.

Alvarez J G and Storey B T (1992) Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethalcryodamage to human sperm during cryopreservation. *Journal of Andrology* **13**(3), 232–241.

Alvarez J G and Storey B T (1992) Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethalcryodamage to human sperm during cryopreservation. *Journal of Andrology* **13**(3), 232–241.

Baril G, Chemineau P, Cognie Y, Guerin Y, Leboeuf B, Orgeur P and Vallet J C (1993) Manuel de formation pour l'inséminationartificielle chez les ovinset les caprins. Rome: FAO: 231p.

Bielañski W, Dudek E F, Bittmar A and Kosiniak K (1982) Some characteristics of common abnormal forms of spermatozoa in highly fertile stallions. *Journal of Reproduction and Fertility Supplement* **32**, 21-6 .

Blom E (1950) A one-minute live-dead sperm stain by means of eosin-nitrogen fertile-steril. **1**, 176-177.

Brody J and Kern S (2004) History and principles of conductive media for standard DNA electrophoresis. *Anal Biochem*. **333**(1), 1-13.

Bruce A, Alexander J, Julian L, Martin R, Keith R and Peter W (2002) Programmed Cell Death (Apoptosis). *Molecular Biology of the Cell*. 4th Edition.

Chella L, Kunene N and Lehloenya K (2017) A comparative study on the quality of semen from Zulu rams at various ages and during different seasons in KwaZulu-Natal, South Africa. *Small. Rum. Res*. **151**, 104-109.

Chenoweth P J (2002) Semen quality assement. In : *Proc. Appl. Reprod.* Strategies in beef cattle workshop. Pp. 247-255.

Daniel W W (1991) Analysis of variance. In: Daniel, W.W. (Ed), *Biostatistic: A Foundation for Analysis in the Health Sciences*. John Wiley & Sons, Hoboken. pp. 74-320.

Elhammali N S A, ALqurashi A M and Elsheikh A S (2013) Puberty of cross breed male goat kids. *J. Anim. Sci.* **9**(4), 9599.

El-Shry M A and Saleh H M (2000) The effect of different level of caged layer droping in male diets on growth performance and and semen characteristics. *Egypt. J. Nutr. Feeds* **3**(1), 1.

Fleming J, Yu F, McDonald R, Meyers S, Montgomery G, Smith J and Nicholson H (2004) Effects of scrotal heating on sperm surface protein PH-20 expression in sheep. *Molecular Reproduction and Development* **68**(1), 103-114.

Foc°ăneanuV, Bogdan L, Andrei S, Bogdan S and Petrean A B (2014) Performance of Some Variables Used as a Procedure for Estimating Sexual Capacity (Fertility) of the Ram. Bulletin of University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca. *Veterinary Medicine* **71**, 52-55.

Ghorbankhani F, Souri M, Moeini M and Mirmahmoudi R (2015) Effect of nutritional state on semen characteristics, testicular size and serum testosterone concentration in Sanjabi ram lambs during the natural breeding season. *Animal Reproduction Science* **153**, 22-28.

Ghozlane F, Ziki B and Yakhlef H (2005) Variations saisonnières des caractèresquantitatifs du sperme de bélier de race Ouled Djellal. *Renc. Rech. Ruminants* **12**, 380.

Gundogan M and Serteser M (2005) Some reproductive parameters and biochemical properties in Akkaraman and Awassi rams. *Turk J Vet Anim. Sci.* **29**, 595-599.

Hafez E (2000) *Reproduction in farm animals* .7th ed. Philadelphia #h Lippincott Williams and Wilkin, p:359.

Hassan M R, Pervage S, Ershaduzzaman M and Talukder M A I (2009) Influence of age on the spermiogramic parameters of native sheep. *J. Bangladesh Agril. Univ*. **7**, 2: 301-304.

Alvarez J G and Storey B T (1992) Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethal cryodamage to human sperm during cryopreservation. *Journal of Andrology* **13**(3), 232–241.

Kelso K A, Redpath A, Noble R C and Speake B K (1997) Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. *J Reprod Fertil*. **109**, 1–6.

Kryndushkin D S, Alexandrov I M, Ter-Avanesyan M D and Kushnirov V V (2003) Yeast (PSI+) prion Aggregates are Formed by Small Sup. 35 Polymers Fragmented by Hsp104. *Journal of Biological Chemistry* **278**(49), 49636–49643.

Hassan M R, Pervage S, Ershaduzzaman M and Talukder M A I (2009) Influence of age on the spermiogramic parameters of native sheep. *J. Bangladesh. Agril. Univ*. **7**(2), 301–304.

Matsuoka T, Hirata M, Tanaka A and Narumlya S (2000) Prostaglandin D2 as A Mediater of Allergic Asthema . Science: 2013 17 March.

Mortimer D (1994) *Practical Laboratory Andrology*. Oxford University Press, New York, USA. pp 66-69.

Okukpe K M, Ologun A G and Alokan J A (2001) Effects of age and collection time on the characteristics of ram semen in Akure, Nigeria. *Bull. Anim. Hlth. Prod. Afr*. **49**, 228-234.

Vernet P, Aitken R J and Drevet J R (2004) Antioxidant strategies in the epididymis. *Molecular and Cellular Endocrinology* 216(1), 31–39.

Mahfouz R, Sharma R and Thiyagarajan A (2010) Semen characteristics and sperm DNA fragmentation in infertile men with low and high levels of seminal reactive oxygen species. *Fertility and Sterility* **94**(6), 2141–2146.

Recabarren S E, Rojas-Garcia P P, Recabarren M P, Alfaro V H, Smith R, Padmanabhan V and Sir-Petermann T (2008) Prenatal testosterone excess reduces sperm count and motility. *Endocrinology* **149**, 6444-6448.

Rege J E O, Toe F, Mukasa-Mugerwa E, Tembely S, Anindo D, Baker R L and Lahlou-Kassi A (2000) Reproductive characteristics of Ethiopian highland sheep. II. Genetic parameters of semen characteristics and their relationships with testicular measurements in ram lambs. *Small Rum. Res*. **37**, 173-187.

Rybar R, Faldikova L and Faldyna M (2004) Bull and boar sperm DNA integrity evaluated by sperm chromatin structure assay in the Czech Republic. *Vet Med*. **49**(1), 1-8

Boussena S, Bouazizi O, Zerrougui S and Derqoui L (2015)

Performances de croissancecorporelleettesticulaireavant le sevrage chez les agneaux de race OuledDjellal. *Revue Méd. Vét*. **164**, 4, 191-199.

Salhab S A, Zarkawi M, Wardeh M F, Al-Masri M R and Kassem R (2003) Characterization and evaluation of semen in growing Awassi ram lambs. *Trop. Anim. Health Prod.* **35**, 455-463.

Salhab S, Zarkawi M, Wardeh M, Al-Masri M and Kassem R (2001) Development of testicular dimensions and size and their relationship to age, body weight and parental size in growing Awassi ram lambs. *Small Ruminant Research* **40**(2), 187-191.

Salisbury G M, Van Denmark N L and Lodge J R (1978) Semen Evaluation.Blom, E. 1950. Interpretation of spermatic cytology in bulls. *Fert. Steril*. **1**(3), 233.

Sambrook J and Russel D W (2001) Molecular Cloning: A Laboratory Manual 3rd Ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Štolc L, Je•ková A, Stádník L and Louda F (2009) Effect of rams' breeds and ages on quantitative and qualitative traits of their sperm. *Výzkum v chovuskotu* **51**, 14–21.

Tabbaa M J, Kridli R T, Al-Ghalban A and Barakeh F S (2006) Age-related changes in scrotal circumference and some semen characteristics in Awassi rams. *Anim. Reprod*. **3**, 431-438.

Toe F, Lahlon-Kassi A and Mukasa-Mugerwa E (1994) Semen characteristics of Ile-De-France rams of different age and physical conditions. *Theriogenology* **42**, 321-326.

Ubah Simon Azubuike, Ogwu David, Rekwot Peter Ibrahim and Rwuaan Joseph Sankey (2017) Specific sperm abnormalities observed in rams (Ovisaries) following cypermethrin treatment. *Journal of Cell and Animal Biology* **11**(1), 1-6,

Van Tran L, Malla B A, Kumar S and Tyagi A K (2017) Polyunsaturated fatty acids in male ruminant reproduction—a review. *Asian-Australasian Journal of Animal Sciences* **30**(5), 622.

Vernet P, Aitken R J and Drevet J R (2003) Antioxidant strategies in the epididymis. *Molecular and Cellular Endocrinology* **216**(1), 31–39. doi: 10.1016/j.mce.10.069.

Yuste V J, Sánchez-López I, Solé C, Moubarak R S, Bayascas J R, Dolcet X, Encinas M, Susin S A and Comella J X (2014) The Contribution of Apoptosis-inducing Factor, Caspase-activated DNase, and Inhibitor of Caspase-activated DNase to the Nuclear Phenotype and DNA Degradation during Apoptosis. *The Journal of Biological Chemistry*.