NTU Journal of Agricultural and Veterinary Science - (NTU-JAVS)
(P-ISSN: 2788-9890 e-ISSN: 2788-9904)

The Effect of Adding Semen Plasma & Some Types of Oils to the Semen Diluents of Awassi Rams on Vitality & Deformitied of Sperm Stored at 5°C for Different Periods

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Received: 10-6-2022, **Accepted:** 16-10-2022, **Published online:** 10-11-2022

Abstract. The current study was conducted to know the effect of adding seminal plasma and olive oil to diluted semen. Twelve Awassi rams were used, given a standard diet, and the diluted semen was divided into three groups; the first group was the control group, the second group was 3.5 ml of semen plasma / 100 ml of diluted semen while the third group had the addition of 3.5 ml of olive oil / 100 ml of diluted semen.

Results obtained from this study indicate that there was a significant effect ($P \leq 0.05$) increasing the percentage of individual motility and live sperm after 96 hours and in reducing the percentage of sperm abnormalities at 72 hours and sperm acrosomal abnormalities at 24 hours when adding semen plasma to sodium citrate diluent in. Futhermore, the addition of olive oil to dilute sodium citrate led to an increase in the percentage of live sperms at all preservation periods and to a decrease in the percentage of sperm and acrosome abnormalities. The storage periods of 48, 72 and 96 hours had a significant effect ($P \leq 0.05$) on the studied traits, as the individual motility of the sperm and the live sperm were decreased with the increase of the preservation period. As for the percentage of sperm abnormalities and acrosome abnormalities of the sperm, it was increased with the progression shelf life at 96 hours compared to 24 hours.

Keywords: Semen Plasma , Oils , AwassiRams.

Introduction

The process of artificial insemination is the most important way to develop the animal production sector and transfer the desired genetic traits, at a time when you need to use advanced techniques in preserving semen and increasing its fertilization capacity. The process of diluting semen helps to prolong life and vitality, because the diluents used to semen had many advantages, including containing nutrients that supply the sperm with energy and containing compounds that protect the sperm from the harmful effects of cooling or freezing (Chimeua et al., 1991). Preservation of semen at 5 °C is considered to be one of the useful methods good for preventing bacterial growth and prolonging the life of the sperm (Morrell et al., 2014). Drabovich et al. (2014) mentioned That seminal plasma is a fluid secrete from the auxiliary glands containing

protein which is important as antibacterial beside containing glutathione peroxidase (GPX) and prostaglandins. The secretions of seminal vesicles and prostate are alkaline and secretion of fructose sugar, the main source of energy (Mann, 1974). Seminal plasma of rams is a complex liquid that helps the sperm in their journey inside the male reproductive system and works to nourish them and provide the necessary energy source for them (Zeny, 2015). The researcher also added that medicinal plant extracts contain antioxidants (Al-Hashimi, 2010). Al-Bazii and Khudiar (2017) noted that olive oil is one of the best types of oils because it contains high amounts of antioxidants . Olive oil, when given in the diet, improves the properties of semen and reduces fat peroxidation (Banihani , 2017). In addition, it is important in feeding roosters of old age to improve the deteriorating and weak semen characteristics (Kacel and Iguer-Ouada, 2018). However, and as a result of the lack of studies in which the processes of adding seminal plasma and olive oil to semen diluents in rams are used, the current study aimed at knowing the effect of adding ram's seminal plasma and olive oil to semen diluents, beside to determining the effect of periods preservation on the activity, vitality and abnormalities of sperm for Awassi rams.

Materials and Methods

The current study was conducted in the fields of the Department of Animal Production - College of Agriculture and Forestry - University of Mosul from the time span of 1/7 to 15/9. Twelve (12) Awassi rams aged 1.5-2 years were used in this study with an average weight of 58.25 kg placed in two chambers. The rough fodder (hay) was provided at a rate of (350) g/animal/day used a standard diet consisting of black barley (55%), wheat bran (33%) and soybean meal (6%). Urea (0.25%), straw (8%), sodium bicarbonate (0.5%), limestone (1%), lime (1%), total protein (14.34) and total metabolic energy (2598.93) kcal/ kg were calculated based on (Al-Khawaja et al., 1978). The semen was collected once every two weeks using an electrical stimulation device (Fourie et al., 2004) to obtain an ejaculation which was collected by a graduated tube, and after the sample collection process. It was placed in a water bath at a temperature of 37°C. The samples with little lone motility were neglected. for sperm in dilute semen as indicated (Evans and Maxwell, 1990). Live and distorted sperm were counted according to (Chemineau et al. 1991) and terminal particle abnormalities were calculated according to (Wells and Awa ,1970). The properties of fresh semen and the most important components of seminal plasma were as shown in table (2) Citrate diluent - egg yolk consisting of glucose 0.5 (g), sodium citrate 2.37 (g) and egg yolk 15 (ml) I use 100,000 IU of penicillin and 100 mg of streptomycin, then adding 100 ml of distilled water according to Salamon and Maxwell (2000). The diluent was divided into three groups: the first was diluted citrate without adding control, the second was diluted citrate adding seminal plasma at a rate of 3.5 ml /100 ml of the diluent (see Table #2) and the third was adding olive oil of a Spanish type (RS) brand at a rate of 3.5 ml / 100 ml of the diluent (see Table #3). A dilution ratio of 16:1 was used and the liquid was stored after diluting the semen for a period of (24, 48, 72 and 96) hours in the refrigerator at 5°C. The total protein concentration in the semenl plasma was measured according to (Tietz ,1986) and the concentration of Albumin was measured according to (Jennifer and Finbarr, 1982) and the level of triglycerides was estimated according to (Fossati and Prencipe ,1982). Reduced glutathione (GSH) was determined using the Elleman reagent method (Al-Hassani, 2005), the enzymatic activity of super oxide dismutase (SOD) as indicated by (Marklund and Marklund, 1974). Statistical analysis was carried out using a one-way complete random design (CRD) according to the following mathematical model Yij=µ+ti+eij (Al-Rawi and Khalaf Allah, 2000) and the differences between the means were tested using Duncan's (1955) polynomial method and using the ready-made statistical program SAS (2000).

Table 1. Fresh semen characteristics in the experiment(mean ± standard error).

Charecterstics	(means ± standard error).
Volume (ml)	60.0±1.68
(٪) Individual motility	0.881 ±83.76
(٪) Live sperm	20.5±89.4
(٪) Abnormal sperm	0.29±.826
pH	0.25±6.83
sperm of Concentration	2.52 ±0.187

Table 2. Semenal plasma of characteristics used in the experiment(mean ± standard error).

Charecterstics	(means ± standard error).
Total protein (gm /100 ml)	0.09±0 3.4
Albumin (gm /100 ml)	0.026±1.48
(gm /100 ml) Glubuline	0.09 ±1.92
(Triglyceride (mg /100 ml	9.33±355.76
Fructose (mg /100 ml)	8.31±0.24
Glutathione reduced (mmol /L)	2.37±14.9
Superoxidedismutase enzyme (u/ ml)	2.5± 134.85

Table 3. The components of olive oil used in the experiment (according to the data of the oil manufacturer(mean ± standard error).

Charecterstics	(means ± standard error).
energy kcal / 100 ml	822
Total lipid(gm /100 ml)	13.65
Saturated lipid(gm /100 ml)	14
Unsaturated primary fatty acids(gm /100 ml)	69
polyunsaturated fatty acids(gm /100 ml)	9.1

Results and discussion

The results in Table #(4) show a significant effect ($P \leq 0.05$) of the treatments group with seminal plasma and olive oil supplemented to semen diluents on the percentage of individual motility of sperms, where the treatment of seminal plasma and olive oil adept at the preservation period of 24 hours compared with the control group, and the treatment of seminal plasma adept in the preservation period of 48 hours compared with the control group. The results show no significant differences between the three treatments after 96 hrs of preservation, The results go with that of AL-

Daraji's (2006) results when adding 2, 4, 6 and 8 ml/100 ml of Spanish olive oil for semen diluent significantly increasing the individual motility of the sperm. Consequently, the current study indicated that adding olive oil improves the semen quality at 5 °C and for 72 hours. It may also be dued to the fact that olive oil reduces the Reactive Oxygen Specious (ROS) in various cellular systems (Dangelo et al., 2001). However, Catt et al. (1997). indicated that adding 10% of the seminal plasma to the diluent increases the individual motility and the percentage of live sperm. The addition of seminal plasma to the diluted semen after thawing had a positive effect on function and fertility of the sperm (Maxwell et al., 1999) whereas the effect of the seminal plasma is through the effect of the protein that binds with the membrane of the ram sperm cell since dilution processes and the components of the seminal plasma are responsible for the function of the sperm (Maxwell, 2006). The fatty components in the semen of rams are also important in reducing the damage of cold shocks and raising the fertile ability. Table #(4) indicates that the percentage of individual movement gradually decreased over the period of preservation for the three transactions, especially between the first period of preservation and the last period of preservation. This result was in agreement with the results of Banana and Al-Khazraji (2017) and Ibrahem (2022) who found a decrease in individual motility of the diluted ram's semen sperm significantly with the progression of the cryopreservation period of 5 m, and the motility of the individual sperms of Balinese bulls stored at 5 °C was decreased after 22 Compared to the beginning of preservation, Cryopreservation and Reactive Oxygen Species (ROS) enhance DNA fragmentation (Maxwell and Stonjano.

Table 4. The effect of treatments and storage period on the percentage of individual sperm motility in the diluted semen (means ± standard error).

TreatmentS	Storage period (hour)				
	24	48	72	96	
T 1	73.400 ±3.878	71.93± 2.19	65.80± 2.168	53.00± 3.67	**
Control	b A	b A	b A	a B	
T 2	81.40 ±2.56	79.00± 1.73	75.400± 1.09	59.55± 3.95	**
Seminal plasma	a A	a A	a A	a B	
T 3	80.00±2.353	76.20± 2.003	70.714± 2.44	62.00± 4.29	**
Olive oile	a A	AB ab	ab B	a C	
	*	**	**	N.S	1.76±78.26

Means with Different letters (a,b,c,d) with each column are differ significantly .
Means with Different letters (A,B,C,D) with each Row differ significantly .
** Significant effect at the level of probability ($p \leq 0.01$).
* Significant effect at the level of probability ($p \leq 0.05$). (N.S) No significant effect.

The deterioration in sperm motility occurs as a result of the shock of cold storage and storage for a long time, and that ROS interacted with unsaturated fatty acids in the sperm membrane and affected its composition, thus causing lipid peroxidation **.** Table #(5) below indicates that there is a significant effect of ($P \leq 0.05$) in the treatments and the preservation period on the percentage of live sperm in the diluted semen of rams, as it was significantly increased in the olive oil and seminal plasma treatment at the storage periods compared with the control group.The high percentage of live sperm in the treatment of seminal plasma may be due to its containing of both vitamins E and C, both of which, in turn, have an antioxidant role and improve fertility parameters (Kammuna et al, 2011). Also the seminal plasma contains potassium ions K + and sodium Na+ which is important in maintaining the osmotic pressure and the work of the necessary enzymes (Zamiri and Khodaei, 2005), and also contains calcium ions, which have a positive effect on sperm vitality (Kareskoski and Katile, 2008). It is important before and after the dilution process, which is responsible for prolonging the freezing period (Moore et al., 2006). This result agrees with AL-Daraji (2006) who obtained high live sperm % of roosters semen preservated with diluent supplemented with olive oil. The antioxidant foods maintained the osmotic pressure and the work of the necessary enzymes (Zamiri and Khodaei, 2005). In addition, olive oil had a moral and positive effect in raising the percentage of live sperm (AL-Daraji, 2006). The effect of added seminal plasma appeared because it contains calcium ions which have a positive effect on sperm vitality (Kareskoski & Katile, 2008). It is important before and after the dilution process which is responsible for prolonging the freezing period (Moore et al., 2006). Antioxidant foods were important to reduce the incidence of oxidative damage to cells which rose with the increasing AST and maintained the cell membrane (AL-Bazi, 2009), and prevented the leakage of enzymes from the cell and scavenges free radicals (Juan et al., 2006). Its

benefits lied in the fact that it contains oleic, followed by linoleic, a little linolenic, and alpha tocopherol (Buckner, 2004).

Table 5. The effect of treatments and storage period on the percentage of live sperm in the diluted semen (means ± standard error).

Treatments	Storage period (hour) 24	48	72	96	
T 1	75.25 ± 1.68	68.57± 3.51	65.00± 3.00	61.57± 2.049	**
Control	c A	b AB	b B	B B	
T 2	84.46 ± 2.06	81.357± 1.47	77.64± 3.28	70.50± 3.37	*
Seminal plasma	b A	a A	a AB	a B	
T 3	87.81± 0.52	83.437± 0.88	79.75± 3.127	72.3± 3.20	**
Olive oile	a A	AB b	a B	a C	
	**	**	**	*	المتوسط العام 78.26

Means with Different letters (a,b,c,d) with each column are differ significantly . Means with Different letters (A,B,C,D) with each Row are differ significantly .
** Significant effect at the level of probability ($p \leq 0.01$).
* Significant effect at the level of probability ($p \leq 0.05$). (N.S) No significant effect.

Table (6) shows a significant effect ($P \leq 0.05$) of the treatments at (48) hours of the storage period which significantly decreased in the seminal plasma treatment to percentage of abnormal sperm compared to the control. The seminal plasma is important in studies inside and outside the animal body and has beneficial effects on the function of the uterus and the mother's immune system (Saccone et al., 2017). Mortimer and Maxwell (2004) said that the addition of seminal plasma to the sperm has simple positive effects in the early stages, but its effects are significant later and AL-Daraji (2006) stated that adding 2, 4, 6 and 8 ml of olive oil/100 ml which led to a significant reduction in the percentage of sperm abnormalities and acrosomal integrity for all storage periods because of olive oil which characterized A and E (Fito et al., 2005). By its solubility, it promotos the spermatic plasma membrane and quenches free radical damage. It contains vitamins on phenolic compounds (Zaher 2008), and selenium which are antioxidants (Al-Mawsili, 2008). Table #(6) indicates that there was a significant effect ($P \leq 0.05$) of the cryopreservation period on the percentage of sperm abnormalities which was increased with the progression of the preservation period. This result agrees with what Al-Sabihawi et al. (2017) stated that the percentage of distorted sperms in the semen of Awassi rams gradually increased and that the effect was significant on the fifth day compared to with the first day due to exposure to cold tempreture.

Table 6. The effect of treatments and storage period on the percentage of abnormal sperm in the diluted semen (means ± standard error).

treatment	Storage period (hour) 24	48	72	96	
T 1	8.5000 ± 0.53	11.27± 0.73	12.33± 0.65	14.66± 0.39	**
Control	a C	a B	a B	a A	
T 2	7.812 ± 1.04	9.50± 1.06	10.12± 0.44	11.33± 0.27	*
Seminal plasma	a B	b AB	b A	b A	
T 3	9.00± 0.76	11.25± 0.90	12.25± 0.33	12.84± 0.618	**
Olive oile	a B	AB a	b A	b A	
	N.S	**	**	*	المتوسط العام 78.26±1.76

Means with Different letters (a,b,c,d) with each column are differ significantly . Means with Different letters (A,B,C,D) with each Row are differ significantly .
** Significant effect at the level of probability ($p \leq 0.01$).
* Significant effect at the level of probability ($p \leq 0.05$). (N.S) No significant effect.

Results in Table #(7) shows a significant effect ($P \leq 0.05$) of the treatments on the percentage of sperm acrosome abnormalities at (48) hours of the storage period, as it was significantly decreased in the treatment of seminal plasma and olive oil compared with the control. Olive oil obtained short-chain saturated fatty acids 8-12% and unsaturated 13 -17 (Hussein et al ,2012) . It was based on the extraction method and contained squalene compounds which are important in

removing free radicals O2- and *OH. and olive oil phenols which have the ability to scavenge free radicals and stop chain reactions Peroxides prevent lipid peroxidation and reduce cell damage (AL-Daraji, 2006). Table #(7) indicates that there was a significant effect (P ≤ 0.05) of the period of cryopreservation on the percentage of sperm acrosome abnormalities which increased with the progression of the preservation period, while differed from what Fernandez-Novo et al. (2021) mentioned that the percentage of acrosome abnormalities of semen diluted by cryopreservation is 5 ˚C. For bulls, it was not significantly affected by the preservation period, so it reached after (2, 6, 24) hours.

Table 7. The effect of treatments and storage period on the percentage of acrosome abnormality of sperm in the diluted semen (means ± standard error).

treatment	Storage period (hour)				
	24	48	72	96	
T 1 Control	10.80 ± 0.11 a D	12.08± 0.09 a C	15.80± 0.33 a B	20.50± 0.33 a A	**
T 2 Seminal plasma	10.33 ± 0.058 a C	10.5± 0.473 b C	13.8± 0.453 b B	17.25± 0.52 b A	**
T 3 Olive oile	9.12± 0.23 a D	9.66± 0.364 C b	12.90± 0.84 b B	15.71± 0.58 c A	**
	N.S	**	**	**	المتوسط العام 78.26±1.76

Means with Different letters (a,b,c,d) with each column are differ significantly . Means with Different letters (A,B,C,D) with each Row are differ significantly .
** Significant effect at the level of probability (p ≤ 0.01).
* Significant effect at the level of probability (p ≤ 0.05). (N.S) No significant effect.

References

[1] Al- Bazii . W . G and K .Khudiar (2017). Preventive Role of Olive Oil On Serum Lipid Profile and Antioxidant Status of Methionine Overload Rabbits (Part II) . Journal of Kerbala for Agricultural Sciences.180-191

[2] AL-Daraji , H. J(2006). Effect of diluent supplementation with olie oil on susceptibility to lipid peroxidation during liquid storage of aged roosters semen .The Iraqi Journal of Agriculture Science: 177-187.

[3] Al-Hashimi, Marwa Abdul-Salam Qadir (2018). Study of the effect of oxidative stress on Athletes in a number of biochemical variables and compare them with the effect of some Plant extracts in reducing stress, Master's thesis, College of Science, University of Tikrit.

[4] Al-Mawsili, Muzaffar Ahmed (2008). Medicinal plants mentioned in the Noble Qur'an and the Prophet's Sunnah, Dar al-Salaam for Printing and Publishing, Mosul..

[5] AL-Rawee , Mahmoud and Abdul Aziz Muhammad Khalaf Allah (2000). Design and analysis of agricultural experiments, Ibn Al-Atheer House for Printing and Publishing, University of Mosul.

[6] Al-Sabihawi, Hawra Resan Bardi and Hazem Kassar Jasir Al-Saab (2017). Extraction and lyophilization of low-density lipoproteins and their use in place of egg yolks in Tris diluents and their effect on some characteristics of the sperm of Awassi rams when kept by cryopreservation at a temperature of 5°C. Iraqi Journal of Agricultural Sciences, 48(1): 350-359.

https://doi.org/10.1016/j.theriogenology.2010.08.016

[7] Banana, Hussam Jassim Hussein and assigning governor Abdul Khazraji (2017). Effect of adding different concentrations of reduced comethathione to diluted semen maintained at 5°C and cryopreservation period on the ratio of single motility and distorted sperm of Awassi rams. Anbar Journal of Veterinary Sciences, 2(10): 87-93. http://www.plantarchives.org

[8] Banihani, S. A. (2017). Semen quality as affected by olive oil. International journal of food properties, 20(sup2), 1901-1906. : https://www.tandfonline.com/loi/ljfp20

[9] Buckner, G. (2004). Microcirculation, vit E and omega -3fatty acids: an overview. Adv. Exp. Med. Biol., 415:195-208.

[10] Catt ,S. L.; O'.Brien.; J.K. Maxwell.; and G. Evans (1997) . Assessment of ram and boar spermatozoa during cell sorting by flow cytometry. Reproduction in Domestic Animals.32 :251-258.

[11] Chemineau, D.; Y. Cogine;Y. Guerin; P. Orgeure and J.C. Valtet (1991). Training manual on Artificial insemination in sheep and goat. FAO. Animal Productive and health, 3: 83- 90. https://cgspace.cgiar.org/bitstream

[12] Dangelo , S.; C.Manna, V.Migliiardi, O.Mazzoni, P.Morrica,G. Capasso. G.Pontoni, P.Galleti and V.Zappia (2001).Pharmacokinatic and etabolism of hydroxytyrosol, a naturaloxidant from olive oil.DMD.29(11):1492-1498.

[13] Drabovich .A. P., P. Saraon.; K. Jarvi and E. P. Diamandis(2014).Seminal plasma as a diagnostic fluid for male reproductive system disorders. REVIEWS. UROLOGY,11:278-299.

[14] Duncan , D.B .K (1955). Multiple range and Multiple F test. Biochemistrics .,(11):1-42.

[15] El-Harairy, M. A.; A. E. Abdel-Khalek.; W. A. Khalil.; E. I. Khalifa.; A. Y. El-Khateeb and A. M. Abdulrhmn (2016). Effect of Aqueous Extracts of Moringa oleifera leaves or Arctium lappa Roots on Lipid Peroxidation and Membrane Integrity of Ram Sperm Preserved at Cool Temperature. J. Anim. and Poultry Prod., Mansoura Univ.7 (12): 467-473. https://dx.doi.org/10.21608/jappmu.2016.48804

[16] Evans, G., and W.M.C.Maxwell, (1990). Salamons Artificial Insemination Of Sheep And Goats. Butterworths, Sydney, Australia.

[17] Fernandez-Novo, A.; S. S. Lopez .; C. B.Masa .; P. Mozas .; E. d. Mercado.; E. Caceres .; A. Garrafa .; J. V.; G.Martin .; N. P.Villalobos .; A. Oliet .; S. Astiz and S. S. Perez-Garnelo (2021). Effects of Extender Type, Storage Time, and Temperature on Bull Semen Parameters, Biology , 10, 630:1-17. https://mdpi-res.com/d_attachment/biology/biology-10-00630/article

[18] Fito, M ; M. Cladellas ; R .Torre and MC Lopez .(2005) Antioxidant effect of virgin olive oil in patients with stable cornary heart disease. Atherosclerosi (58) : 181-202. https://doi.org/10.1016/j.atherosclerosis.2004.12.036

[19] Fossati, P. and Prencipe, L . (1982) . Serum Triglycerides determination colorimtrically with an enzyme that produces hydrogen peroxides. Clin Chem. 28 : 2077-2085.

[20] Fourie, P. J., L. M. Schwalback, F. W. C. Neser and C. Vander Westhuizen (2004). Scrotal testicular and semen characteristics of young Dorper rams managed under intensive and extensive condition. Small Rumin. Res. 54:53-59. https://doi.org/10.1016/j.smallrumres.2003.10.011

[21] Hussein, Faryal Farouk, Saadat Mustafa Muhammad and Nour Juma Faad (2012). The effect of olive oil and black seed Nigella sativa L, Linumusitatissimum L and butter in healthy subjects' blood lipid levels. Mesopotamia Journal of Agriculture 20 (12): 146-152.

[22] Ibrahem, M, S (2022). Effect of some Antioxidant Substanses and The Seasons of the Year on the Reproductive and Histological Parameter of Awassi Rams. Ph.D Thesis College of Agriculture and Forestry, University of Mosul

[23] Jennifer, D. and Finbarr, D. P . (1982) . Albumin by bromocresol green- a case of laboratory conservatism. Clin. Chem., 28(6): 1407-1408.

[24] Juan, M.;Wenzel, U.;Ruiz-Gutierrez,H.;Daniel,A. and Planas,M.(2006).Olive Fruit Extract Inhibit Proliferation and induce Apoptosis in HT-29 Human Colon Cancer Cell. J. Nutr., 136(10):2553-2557. https://doi.org/10.1093/jn/136.10.2553

[25] Kacel, A., & Iguer-Ouada, M. (2018). Effects of olive oil dietary supplementation on sperm quality and seminal biochemical parameters in rooster. Journal of animal physiology and animal nutrition, 102(6), 1608-1614. https://doi.org/10.1111/jpn.12983

[26] Kammuna, Zainab Hekmat Abd and Sahib Ali Mahdi Hadi Rasul Hassan Al-Masoudi (2011). Study the relationship between the concentrations of sperm antibodies and both oxidative stress and antioxidants in the seminal plasma of non-fertile men, and. Karbala University Scientific Journal. 9 (4): 185-192.

[27] Kareskoski .M and T. Katila (2008).Components of stallion seminal plasma and the effects of seminal plasma on sperm longevity. Animal Reproduction Science 107 (2008) 249–256. https://doi.org/10.1016/j.anireprosci.2008.04.013

[28] Mann, T (1974) .Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. *J. Reprod. Fertil.* 37, 179–188. https://doi.org/10.1530/jrf.0.0370179

[29] Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Europ. J. Biochem., 47: 469-474.

[30] Maxwell ,WMC.and Stojanov,T.(1996). Involvement of the superoxide anion radical in the autoxidation of. Reprod Fertile Dev . 1013: 8-20.

[31] Maxwell .W.M.C and L.A .Johnson (1999).Physiology of spermatozoa at high dilution rates: The influence of seminal plasma. Theriogenology52 1353-1362. https://doi.org/10.1016/S0093-691X(99)00222-8

[32] Maxwell .W.M.C.; S.P.de Graaf.; R. E. Ghaoui and G. Evans (2006). Seminal plasma effects on sperm handling and female fertility.13-39.Corresponding author E-mail: chism@vetsci.usyd.edu.au

[33] Moore .A.l.; E.L. Squires and J.K. Graham (2005).Effect of seminal plasma on the cryopreservation of equine spermatozo .Theriogenology. 63 2372-2381. https://doi.org/10.1016/j.theriogenology.2004.05.032

[34] Morrell, J. M.; Klein, C.; Lundeheim, N.; Erol, E. & Troedsson, M. H. T. (2014). Removal of bacteria from stallion semen by colloid centrifugation. Anim. Reprod. Sci., 145(1-2): 47-53. https://doi.org/10.1016/j.anireprosci.2014.01.005

[35] Mortimer.S.T and W.M.C. Maxwell (2004).Effect of medium onthe kinematics of frozen-thawed ram spermatozoa. Reproduction127 285-291. https://doi.org/10.1530/rep.1.00075

[36] Muhammad, Salih Muhammad, Saeb Younes Abd al-Rahman and Osama Ibrahim Azzawi (2018). Improving the semen characteristics of Shami goats outside and inside the reproductive season by adding antioxidants. Tikrit University Journal of Agricultural Sciences, 18(2): 192-200. http://www.plantarchives.org/SPL%20ISSUE%2020-2/652__3959-3964_.pdf

[37] Saccone,G .; A. D. S. Sardo.; B. A. Ciardulli.; C. Caissutti.; M. Spinelli.; D. Surbek and M. Wolff

(2017). . Effectiveness of seminal plasma in in vitro fertilisation treatment: a systematic review and meta-analysis An International Journal of Obstetrics and Gynaecology.220-226. https://doi.org/10.1111/1471-0528.15004

[38] Salamon, S.; W.M.C. Maxwell(2000). Storage of ram semen. Anim. Reprod. Sci. 62: 77-111. https://doi.org/10.1016/S0378-4320(00)00155-X

[39] SAS (2000). Statistical analysis system SAS/ATAT user guide for personal computer . Release 6. 12 . SAS Institute Inc . Cary , NC https://doi.org/10.1590/S1413-70542011000600001

[40] Tietz, N. W. (1986). Textbook of Clinical Biochemistry. W.B. Saunders Co.,Philadelphia, USA, p. 135.

[41] Wells, M. E , . and A wa , O.A.(1970). New Technique for Assessing Acrosomal Characterstics of Spermatozoa .J.Dairy.Sci.53(2). https://doi.org/10.3168/jds.S0022-0302(70)86184-7

[42] Zaher , K .; Ahmed , W.M and N.S, Zerizer , (2008) observation on the biological effects of black cumin seed (*Nigella sativa l.*) and green tea (*camellia sinensis*) Global Veterinaria 2 (4) : 198-204. © IDOSI Publications, 2008

[43] Zamiri M.J and H.R. Khodaei (2005). Seasonal thyroidal activity and reproductive characteristics of Iranian fat-tailed rams. Animal Reproduction Science. 88(3-4): 245-255. https://doi.org/10.1016/j.anireprosci.2004.12.005

[44] Zeny, Z. A. H. T (2015). Effect of different levels of vitamin E in some semen Biochemical Parameters in Awassi rams. Kufa Journal For Agricultural Sciences.7(4):256-263.