



STUDYING THE RELATIONSHIP BETWEEN SEMINAL PLASMA ENZYMES AND CHEMICAL ELEMENTS CONCENTRATION WITH THE PHYSICAL SEMEN PROPERTIES OF LOCAL AWASSI RAMS

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ABSTRACT

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Semen samples were collected by using ten adult Awassi Rams biweekly via electric ejaculator in one ejaculate/ram. Semen properties, enzyme concentration (AST, ALT, ALP, LDH, ACP), and element concentration (Na, K, Ca, Zn, Cl, P, Mg, and Fe) in semen were determined. The results showed that AST enzyme is significantly correlated ($r = -0.671$), ($r = -0.500$), ($r = -0.587$), ($r = 0.587$) with pH, individual motility, live and dead sperms. ALT enzyme was significantly correlated ($r = -0.578$), ($r = -0.560$), ($r = 0.560$) with pH, live and dead sperms respectively. A negative relationship was found between ALP and pH, mass and individual motility, live sperm, semen volume and sperm membrane integrity. While, there was a positive relationship between sperm concentration and dead and abnormal sperm. LDH enzyme positively correlated with, mass and individual motility, semen volume, live and abnormal sperms while negatively correlated with pH, sperm concentration, dead sperm, and sperm membrane integrity. ACP enzyme positively correlated with volume, mass and individual motility, dead sperms, sperm concentration, and sperm membrane integrity. Na and Cl were correlated significantly with semen volume and Cl with mass motility. Zn, Ca, Mg, and Fe were positively and significantly correlated with sperm concentration. On the other hand, Ca correlated significantly with individual motility and live and sperm concentration, while this correlation was significantly negative with dead and abnormal sperm. Therefore, the status of the enzymes and minerals of seminal fluids is considered as important metrics for assessing the quality of Awassi rams semen.

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INTRODUCTION

The process of evaluating semen plasma components and their quality has become the objective of many studies in recent years. Amongst these studies is the study of the enzymatic level in semen plasma to determine its quality (Dhami and Kodagali, 1990). Semen plasma is a complicated mixture of epididymal secretions and subsequent accessory gland secretions, and these glands differ in their chemical composition and secretory functions in mammalian species (La Falci *et al.*, 2002). Many enzymes in the semen plasma are essential for the metabolic processes of sperm in addition to its function. Several researchers studied the level of these enzymes represented by the Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Acid phosphatase (ACP) in various animals to investigate the relationship between their levels and male

fertility or to find a specific correlation between the concentration of these enzymes and the properties of semen. Researchers concluded that these enzymes have a significant correlation with the nature of the sperm cell, the metabolic reactions of the sperm, and the amount of damage and death of spermatozoa in semen plasma (Okab, 2007). It is known that these enzymes penetrate the sperm cell membrane and exit into the seminal plasma fluid when sperm damage occurs, as the study of (AST) and (ALT) activities in semen plasma is the best evidence of semen quality, the status of spermatozoa and its function (Raouf and Taha, 2021) and a measure of the stability of the typical shape of the sperm membrane (Corteel,1980). Thus, the process of releasing (AST and ALT) enzymes from spermatozoa to extracellular fluid has to do with the permeability of sperm membrane, and this process of releasing is caused by the breakdown of the spermatozoa plasma membrane or spermatozoa caps hydrolysis or spermatozoa damage (Bower *et al.*,1973). (Gundogan, 2006), found an inverse correlation between AST and ALT enzymatic activity with individual and mass motility and spermatozoa concentration and a positive relationship with sperm abnormalities. (Gundogan, 2006) and (Pesch *et al.* 2006) stated that LDH enzyme positively correlated with individual and progressive motility, live sperm, semen volume, and sperm concentration. The increase in dead sperm percentage led to a reduction in LDH levels in semen plasma, which indicates that this enzyme is involved in the metabolic reactions of sperm (Duan and Goldberg, 2003). Furthermore, there was a negative correlation between LDH enzyme and dead sperm. Dogan (2009), also observed a significant inverse relationship between volume of ejaculate and LDH enzyme activity and a positive significant relationship between ALP activity and sperm concentration and total sperm count, while the researcher found a negative relationship between ACP enzyme level and sperm concentration. Alfonso *et al.* (2013), found that ALP activity significantly correlated with sperm motility and sperm concentration. Also, he observed a negative relationship with ejaculate volume and stated that this enzyme is essential for the maturation of spermatozoa during ejaculation. Mineral elements for instance Na, K, Ca, P, Zn,Mg, and Fe are some of the leading chemical components of semen, which are secreted from the accessory glands in semen fluid (El Zbieta *et al.*, 2022). These elements maintained the osmotic balance of seminal fluid plasma, and it is necessary for the metabolism, spermatozoa production and maturation, as well as the sperm capacitation and sperms motility in semen plasma (Mirnamniha *et al.*, 2019). The ionic environment in semen plasma determines the activity of spermatozoa, which differs among individuals and species (Kareskoski and Katila,2008). Furthermore, differences in the concentration of these elements in semen plasma may lead to a decline in animal fertility and semen quality (Azab *et al.*, 2021 and Akpa *et al.*, 2013). It has been noted that K and Na have a relationship with the functional activity of spermatozoa and the reaction of the sperm acrosome (Mirnamniha *et al.*, 2019). Also, Abdel-Rahman *et al.* (2000) indicated that the relationship between K element and sperm concentration and vita ejaculation vitality plays a role in maintaining the osmotic pressure in the sperm cell membrane in semen fluid plasma.

Moreover, the high level of Na concentration in the extracellular may lead to improve the motility of spermatozoa (Kong *et al.*, 2009 and Holt and Harrison, 2002). Akpa *et al.* (2013) indicate positive relationship between Na and semen volume,

individual motility of sperms in males' semen and goats' negative, and correlation with the pH of semen. Calcium (Ca) is an essential element in maintaining the effectiveness of spermatozoa acrosome and the activity of spermatozoa motility (Kumar et al., 2011). Also, (Barrier - Battut *et al.*, 2002 and Ali *et al.*, 2019) pointed out that Iron (Fe), Zinc (Zn) elements are important components of seminal plasma, in addition to their antioxidant qualities and ability to improve immune activities (Abbi, 2024). In addition, Zn plays involved in several stages of spermatogenesis and also involved in the physiological function of spermatozoa. Alfonso *et al.* (2013) reported high positive relation between Zn ion and spermatozoa motility, vitality, and sperm concentration. Also, they observed invers relation between Zn ion and spermatozoa membrane integrity and abnormal sperm phenotype.

To our knowledge, few information about the relationship of enzymatic activities, elements, and semen qualities of Awassi rams. Therefore, this study aimed to estimate the enzymatic activities and the chemical content of chemical elements in the semen plasma, which are regarded as good indicators of semen quality. This study also conducted to investigate the relationship between these enzymes, the chemical elements, and semen's physical properties.

MATERIALS AND METHODS

Ethical approval

The study and samples collection were carried out with the agreement of the ethical and animal welfare committee under the number Um. Vet.2023.048 at 15/5/2023 of the College of Veterinary Medicine, University of Mosul.

Experimental Animals

During the breeding season, sixty ejaculations of semen were collected from 10 Awassi rams, 3 years of age, 53 kg average live body weight. The rams were taken from the field of the Animal Production Department of the College of Agriculture and Forestry / Mosul University. Rams were kept in the same breeding condition and basal diet, consisting of mixed hay as roughage and concentrate feed.

Semen collection and Evaluation

Semen ejaculations were taken from the experimental rams twice a month (every 15 days) using a Bailey-type electric ejector device for sheep and goats (Hameed and Alkhashab,2019), using the method of Silva *et al.*(2024). The experiment lasted for three months. After the collection process, semen samples were kept in a water bath at 37 C°, and the sub-sample was evaluated for the physical properties of the semen. Volume of each ejaculate was estimated with the inserted collection tube. pH was measured using pH meter device. Also, mass motility was measured as indicated by Campbell *et al.* (2003). Individual motility, sperm cell concentration, and the percentage of sperm abnormalities were measured according to Evans and Maxwell, (1987). The rate of the live and dead sperms were measured by the method of Swanson and Bearden (1951). The integrity of the sperm membrane was measured using the hypo-osmotic swelling test (HOST), by taking 100 microliters of raw semen and adding it to 1 ml of osmotic solution of fructose-sodium citrate (150 mmol / L), then incubating it at a temperature of 37 C° for 60 minutes. An electronic microscope was used, and at least 200 spermatozoa were noted at a magnification power of × 400. The number of swelling sperms and sperms membrane

integrity were recorded. Also, the percentage of swelling sperms rate were monitored by dividing their number by the total spermatozoa count $\times 100$, and all these values were recorded for each one of the samples.

Semen plasma and Evaluation

After obtaining the physical measurements of semen, a centrifuge was used at 3000 rpm for 15 minutes to get seminal plasma. After separating seminal plasma, it was stored at -20 C° till determination of chemical properties of enzymes and elements were measured later. Enzyme measurements were performed for semen plasma, (AST) and (ALT) enzymes measured according to (Frankel and Reitman,1957), (ALP) according to King and King (1954), (LDH) and (ACP) measured according to Henry (2001) and (Tietz,1999) respectively. The elements (Na, K, Ca, Cl, Zn, P, Mg, and Fe) were measured using an automatic analyzer and assay kits. All measurements of mineral elements were done in the central laboratory, College of Agriculture and Forestry, Mosul University.

Statistical analysis

The collected data were analyzed by using Pearson’s correlation coefficients using SAS, (2018) to study the relationship between the enzyme activities, chemical elements, and the physical properties of the semen. The correlation was found to be significant at a likelihood level of $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Table (1) indicates the results of the enzymatic activities, elements and physical properties of semen.

Table (1): Overall means values of seminal components including enzymes, chemical elements and semen physical properties of Awassi rams

Semen Physical properties	Semen components				
	Means	Enzymes	Means	Elements	Means
Volume (ml)	1.38	AST(U/l)	202.66	Na mmol/L	150
pH	6.7	ALT(U/l)	94.64	K mmol/L	11.61
Mass Motility (1-5)	3.64	ALP(U/l)	2673.16	Ca mg/dL	4.145
Individual Motility (%)	84.1	AcP(U/l)	347	Zn mg/dl	3.299
Conc. Sperm $\times 10^9$	3.12	LDH(U/l)	121.33	CL mmol/dL	18.482
Live Sperm (%)	84.55			P mg/dL	19.26
Dead Sperm (%)	15.44			Mg mg/L	1.875
Abnormal Sperm (%)	8.29			Fe mg/L	1.16
Sperm membrane integrity (Host) (%)	81.33				

Correlation between semen enzymes activity and seminal properties

Results listed in Table (2), concerning the relationship between semen enzyme activities and the semen's physical properties indicated that there is a negative significant ($p \leq 0.01$) correlation between AST and ALT enzymes on the one hand and the pH of semen plasma on the other hand. An important ($p \leq 0.05$) negative relationship exists between mass motility, individual motility, and live sperm. A significant positive with dead and abnormal sperm percentage. In contrast, the

relationship between the AST and ALT was positive with the ejaculate volume. These results agreed with the observation of Dogan *et al.* (2009), who stated that there is a positive relationship between AST enzyme and the volume of the ejaculate. Also, the result agreed with Gundogan (2006), who recorded a negative correlation between the level of the AST enzyme with mass and individual motility and sperm cell concentration in semen Akkaraman and Awassi rams. Similar pattern reported by Frydrychovas *et al.* (2015), who obtained a significant correlation coefficient for AST enzyme activity in seminal plasma with semen volume in male boars, and in contrary the researcher obtained an appositive correlation with individual motility and morphological abnormal spermatozoa.

The results in current study showed a significant negative ($p \leq 0.05$) correlation relationship between ALT activity with live sperms and a positive significant ($p \leq 0.05$) relationship with dead sperms. However, non-significant ALT activity correlated negatively with mass motility, individual motility, and Sperm concentration ($r = -0.180$) ($r = -0.391$) ($r = -0.042$), respectively. This is in agreement with Raouf and Taha (2021) and Azawi *et al.* (1990), who noted an inverse relationship between the activity of the ALT enzyme and both the individual motility and concentration of sperms, and positively correlated with the percentage of abnormal sperms. Our results are agreed with Thirumala *et al.* (2017), who noted a positive correlation between AST, ALT, and AcP enzymes with semen volume, while our finding disagreement with Alfonso *et al.* (2013), who recorded a negative correlation between ALT enzyme compared with semen volume of male pigs. The high levels of these enzymes (AST and ALT) in the seminal plasma means a high number of dead spermatozoa and the breakdown of spermatozoa membranes, which causes the depletion of enzymes (Taha *et al.*, 2000). There is a relationship or effect of AST and ALT enzymes with fertility and the process of spermatogenesis (Pace and Graham, 1970), and poor-fertility semen showed significantly higher levels of AST enzyme concentration in semen plasma (Pace and Graham, 1970).

Table (2): The correlation between semen enzymes activity and seminal properties in Awassi rams.

Semen enzymes	Vol.	pH	Mass Mot.	Indiv. Mot.	Sperm conc.	Live sperm	Dead sperm	Abn. Sperm	Sperm membrane integrity (Host)
AST	0.148	-0.671**	-0.253	-0.500*	-0.074	-0.587*	0.587*	0.338	-0.360
ALT	0.174	-0.578**	-0.180	-0.391	-0.042	-0.560*	0.560*	0.275	-0.337
ALP	-0.355	-0.166	-0.233	-0.177	0.412	-0.341	0.341	0.156	-0.342
ACP	0.126	-0.324	0.159	0.152	0.086	-0.094	0.094	-0.170	0.048
LDH	0.330	-0.467	0.020	0.008	-0.242	0.214	-0.214	0.137	-0.211

*Correlation is significant at $p \leq 0.05$

** correlation is significant at $p \leq 0.01$

The results revealed a positive, non-significant correlation between the ALP enzyme and sperm cell concentration, dead and sperm abnormalities percentage. At the same time, the relationship was negative with the other physical parameters of semen. This finding agrees with the observation of (Dogan, 2009), who found a positive correlation between the level of ALP enzyme and sperm concentration in the

semen of Arabian horses. (Thirumala *et al.*, 2017), also observed an inverse correlation between ALP enzyme with semen volume, sperm concentration and spermatozoa motility. An inverse correlation was recorded between the activity of the ACP enzyme and semen pH and live and abnormal sperm. At the same time, it was positive with the other semen parameters. As for the LDH enzyme, the results in Table (2) showed a positive correlation with semen volume, mass and individual motility, live sperm and abnormal spermatozoa, while the relationship was negatively correlated with the other physical semen parameters. This finding indicates that the LDH enzyme enters into the metabolic reaction of spermatozoa. The activity of this enzyme can be a sign of the metabolic activity of sperm and an increase in concentration of this enzyme in semen plasma (Duan and Goldberg., 2003). It can be used to indicate less sperm cell membrane integrity (Dube *et al.*, 1982). Also, results in agreement with (Pesch *et al.*, 2006), which found a positive correlation between LDH concentration with individual and progressive motility and alive spermatozoa. This result also agreed with that reported by (Asadpour, 2012), who found a highly significant correlation between LDH enzyme and spermatozoa vitality and that an increase in the level of LDH in semen plasma offsets the increase in the level of live and normal spermatozoa. Similar to our results Viudes-de-Castro *et al.* (2015), observed inverse relationship between LDH with sperm concentration, while the results did not agree with his finding in the ties of LDH enzyme with the ratio of abnormal spermatozoa, also the results of this study in contrast with (Dogan *et al.*, 2009), which obtained a significant negative correlation ($r = -0.65$) between LDH and semen volume. Still, our results agree with the researchers' finding of an inverse relationship between ACP and pH and a positive relationship with sperm concentration.

The results also showed an inverse correlation between the (HOST) hypo-osmotic swelling test, which expresses the spermatozoa membranes integrity with AST, ALT, ALP, and LDH enzymes. Simultaneously, a marginally favorable correlation was found with the ACP enzyme. The HOST test is utilized to determine the quality of sperm by assessing the spermatozoa membrane integrity in animals, which is also considered an indicator of the ability of sperm to fertilize; Because of its close relationship to semen properties, this test is frequently utilized as a crucial criterion in the evaluation of semen (Zubair *et al.*, 2015). Dhama and Kodagali (1990) have stated that the process of releasing these enzymes (AST, ALT) to semen plasma due to the breakdown of the sperm cell membrane or hydrolysis in sperm acrosome or the death of spermatozoa. The present results in agreement with that indicated by (Umar *et al.*, 2017), who observed a negative relation between AST enzyme and intact spermatozoa.

Correlation between semen elements concentration and seminal properties

The results in Table (3) showed that Na mineral concentration significantly ($p \leq 0.01$) and positive correlated ($r = 0.623$) with ejaculate volume and insignificantly and positively with mass and individual motility and live sperm percentage. In contrast, the relationship was negative and non-significant with PH, sperm concentration, dead sperms, and sperm membrane integrity. This finding in agreement with (Akpa *et al.*, 2013), which indicated a positive correlation between Na with semen volume and individual spermatozoa motility in the semen of male

goats and negatively correlated with pH. The results also consistent with (Alfonso *et al.*, 2013), who reported that Na concentration positively correlated with semen volume, while the researcher received a negative correlation with individual sperm motility, live spermatozoa, sperm concentration, and sperm membrane integrity. Also, (Abdel-Rahman *et al.*, 2000) obtained negative correlation in semen plasma of rams between Na with sperm concentration and live sperm ratio and positively Na correlated with spermatozoa motility. While the results did not agree with his results in obtaining a negative correlation between Na and ejaculate volume, also our results agreed with (Umar *et al.*, 2017), which reported that Na concentration negatively correlated with percentage of dead sperms ($r = -0.278$). The results showed positive and non-significant correlation between K and semen volume, mass and individual spermatozoa motility, live sperm, sperm concentration, abnormal sperm percentage, and spermatozoa membrane integrity. The relationship was negative with the rate of dead sperm. The potassium K mineral act a major role in the hyperpolarization of the sperm's cell membrane, and this hyperpolarization is necessary to take advantage of the stored calcium in the reaction of spermatozoa head (acrosome). The hyperpolarization of the spermatozoa membrane, which depends on the activity of K within the potassium-calcium channel.

Table (3): The correlation between semen elements concentration and seminal properties in Awassi rams.

Semen elements	Volume	pH	Mass Mot.	Indiv. Mot.	Sperm Conc.	Live sperm	Dead sperm	Abn. Sperm	Host
Na	0.623**	-0.195	0.179	0.015	-0.298	0.227	-0.277	0.008	-0.236
K	0.466	0.160	0.341	0.128	0.381	0.045	-0.045	0.011	0.060
Ca	0.265	0.299	0.333	0.473*	0.605**	0.590**	-0.590**	-0.553	0.358
Zn	-0.495*	0.260	-0.060	0.055	0.622**	0.294	-0.294	-0.166	0.080
Cl	-0.838**	-0.067	-0.608**	-0.198	-0.033	-0.088	0.088	0.296	-0.160
P	-0.181	0.143	-0.233	-0.184	-0.707**	-0.079	0.079	0.043	-0.190
Mg	-0.328	0.186	-0.207	0.129	0.878**	0.106	-0.106	-0.190	-0.037
Fe	-0.185	0.442	0.031	-0.123	0.812**	0.095	-0.095	-0.106	-0.016

*Correlation is significant at $p \leq 0.05$

** correlation is significant at $p \leq 0.01$

Which has a fundamental role in regulating the biological events that lead to the reaction of the acrosome and, therefore, has an essential role in fertilization (Rossato *et al.*, 2001 and Lianos *et al.*, 1994). The current results agree with Abdel-Rahman *et al.*, (2000), who indicated a positive relationship between K concentration with ejaculate volume, sperm concentration, percentage of live sperm, motility and sperm concentration in ram semen, the results also in agreement with Alfonso *et al.*, (2013), who observed a positive relationship of K with individual motility, sperm concentration, live sperm and cell membrane integrity, contrary the researcher obtained a negative correlation relationship with ejaculate volume, also agreed with (Akpa *et al.*, 2013), who reported that K correlated positively with semen volume, individual motility and sperm concentration. In addition to, the researcher indicated that sperm motility activity can be improve or increase with higher concentrations of K and Na in semen, Sodium and potassium are responsible for maintaining osmotic balance in the seminal plasma and are mainly responsible for activating sperm

motility, and reducing in the levels of these two elements is related to a reduction in sperm motility in semen (Zamiri *et al.*, 2010), in contrast (Kaya *et al.*, 2002) obtained inverse correlation between K and Na concentration with sperm motility in ram semen.

The current study revealed significant ($p \leq 0.05$) positive relationship between Ca concentration with semen volume, mass, and individual motility and high significant ($p \leq 0.01$) positive relationship with sperm concentration and live sperm percentage. In contrast, the relationship was substantial ($p \leq 0.05$) and negative with abnormal sperm and highly significant ($p \leq 0.01$) and negative with dead sperm percentage. The present results in agreement with Akpa *et al.*, (2013), who indicated a positive correlation between Ca with ejaculate volume, sperm concentration and individual motility, the researcher pointed out that an increase in ejaculate volume leads to a higher concentration of Ca and K elements in semen plasma. These results agreed with (Alfonso *et al.*, 2013), who observed a positive correlation between Ca with the individual and progressive motility of sperm, live sperm, sperm concentration and sperm membrane integrity, while he obtained inverse correlation with ejaculate volume. Our result also agrees with (Kanwal *et al.*, 2000), who reported a positive relationship between Ca concentration and semen ejaculate volume of bulls. (Abdel-Rahman *et al.*, 2000) also found significant relationship between Ca concentration with live sperm and sperm concentration. In contrast, the results did not agree with obtaining a negative relationship between Ca and sperm motility, while the results agreed with (Ghaniei *et al.*, 2018), who indicated that Ca correlated positively with sperm motility and sperm vitality in Roosters. The current results also in agreement with (Thirumala *et al.*, 2017), which obtained non-significant positive correlation between calcium concentration with PH of semen, sperm concentration, sperm cells membrane integrity, and a significant positive correlation with sperm motility.

Researchers have suggested that Ca concentration regulate the adaptation and hyperactivity of sperm by controlling the availability of ATB inside sperm cells, and the high level of Ca may lead to an increase in the external secretions of the acrosome of sperms and thus may lead to a decrease the fertility (Rossato *et al.*, 2001). With regards to the Zn element, the results pointed out invers and significant ($p \leq 0.05$) correlation between Zn and ejaculate volume, a highly significant positive correlation ($p \leq 0.01$) between Zn with sperm concentration, and a positive correlation with pH, individual motility, live sperm, and sperm cell membrane integrity. Meanwhile, the relationship between Zn with mass motility and the ratio of dead sperm was negative. Similar finding observed with (Alfonso *et al.*, 2013), who obtained non-significant negative correlation between Zn with volume of semen and the ratio of abnormal sperm. Conversely, a high rate of abnormal sperm may lead to intracellular Zn accumulation, decreasing its level in the semen plasma (Westmoreland *et al.*, 1967). In contrast, this results were inconsistent with (Alfonso *et al.*, 2013) in obtaining a negative correlation between Zn and sperm membrane integrity, while the results agreed with his finding for a positive correlation of Zn with spermatozoa motility, sperm vitality, and sperm concentration, and the lower of Zn level in seminal plasma associated with lower in motility, sperm vitality, and infertility (Chia *et al.*, 2000). Also the results in agreement with (Michal *et al.*, 2019), who obtained positive

and significant correlation between Zn concentration and sperm concentration. A high significant ($p \leq 0.01$) negative correlation was observed between chlorine Cl with semen volume and mass motility and a non-significant negative correlation with individual sperm motility, Live sperm, sperm concentration and sperm membrane integrity. At the same time, the relationship was positive and non-significant with the ratio of dead and abnormal sperm. The results agree with (Umar *et al.*, 2017), who reported that Cl concentration significantly and negatively correlated with mass motility and a negative correlation with individual motility, live sperm, sperm concentration, and sperm membrane integrity in male goats' semen. Also, the results in agreement with (Abdel-Rahman *et al.*, 2000), who recorded a non-significant negative correlation between Cl concentration with the ratio of live sperm and significantly and negative correlation with sperm concentration, while the results did not agree with his obtaining a positive correlation between Cl content in seminal plasma fluid with semen volume and spermatozoa motility. In the same trend, (Alfonso *et al.*, 2013), obtained a negative correlation between Cl with ejaculate volume of male boars and a negative significant correlation with intact sperm and sperm membrane integrity. From our study, this inverse relationship between Na and Cl with healthy and normal sperm may be due to the leakage of abnormal spermatozoa into the seminal fluid and, thus, a change in the level of Na and Cl, which is necessary for the metabolism of sperm (Alfonso *et al.*, 2013).

The phosphorus (P) element concentration was negatively correlated and highly significant ($p \leq 0.01$) with sperm concentration, positively and non-significant with pH, dead and abnormal spermatozoa, and negative with semen volume, mass, and individual motility, live sperm, and sperm membrane integrity. The results agree with (Abdel-Rahman *et al.*, 2000), who found a significant negative correlation between P concentration with sperm concentration and a negative non-significant correlation with live sperm. Still, on the contrary, they obtained a positive correlation with spermatozoa motility. Our results in agreement with those of (Umar *et al.*, 2017), which obtained a significant and negative correlation between P and sperm concentration in semen ejaculate of teddy goats.

Table (3) demonstrated significantly ($p \leq 0.01$) higher positive correlation between Mg concentration with sperm concentration and a non-significant positive correlation with PH, individual motility and live sperm. Non-significant and negative correlation existed with ejaculate volume, mass motility, dead and abnormal sperm, and membrane integrity. This finding agreed with (Abdel-Rahman *et al.* 2000), who recorded a significant positive correlation between Mg with sperm concentration and a non-significant positive correlation with spermatozoa motility and live sperm. This results in contrast with those of (Michal *et al.*, 2019), who pointed out a significant positive correlation between Mg concentration and semen volume. In our study, Fe element in semen plasma showed positive correlation and a highly significant ($p \leq 0.01$) ($r=0.812$) with sperm concentration, while, non-significant and negative correlation was obtained with semen volume, individual sperm motility, dead and abnormal sperm, and sperm membrane integrity, and non-significant and positively correlated with pH, mass motility, and live sperm. The results agree with (Amir *et al.* 2021), who found a negative, non-significant correlation between Fe element concentration and semen volume and a non-significant correlation with sperm

motility. These results in agreement with (Hashemi *et al.*, 2018), which indicated that high concentration of Fe in semen was correlated negatively with spermatozoa motility, and perhaps the high level of Fe may inhibit sperm motility by increasing fat oxidation and increasing the MDA compound in semen (Huang *et al.*, 2001). In contrast, (Akalina *et al.*, 2015), recorded non-significant correlation between Fe with semen volume and individual motility in semen of Marino Rams, while the results agree with his obtaining a positive correlation between Fe with sperm concentration and sperm vitality. On the contrary, (Amir *et al.*, 2021) received a significant negative correlation of Fe with sperm concentration and a positively correlated with the ratio of abnormal spermatozoa. (Akalina *et al.*, 2015) also obtained a positive correlation between the Fe with the percentage of sperm membrane integrity and abnormal spermatozoa in ram semen.

The differences between some researchers results and the current study are probably due to the type of animal, age, nutrition and season (Alkass and Mustafa, 2023 and Abdullah *et al.*, 2022), the method of collecting semen samples and number of semen ejaculations taken. In addition to testosterone hormone concentration, which correlated with semen quality (Sabah *et al.*, 2011).

CONCLUSIONS

The analysis of biochemical components such as enzymatic activities and the concentration of elements can be a basic indicator for evaluation the quality of semen in Rams. From the results of the current study, it was indicated that there were correlations between the activity of enzymes and the content of seminal fluid of mineral elements, chemical elements and the semen's physical properties in Awassi rams. More studies must be done about the relationship between semen properties and semen fluid content of bucks.

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CONFLICT OF INTEREST

The authors report no conflicts of interest, and they are responsible for the content and writing of the paper.

دراسة العلاقة بين إنزيمات البلازما المنوية وتركيز العناصر الكيميائية مع الخصائص الفيزيائية للسائل المنوي للكباش العواسية المحلية

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الخلاصة

تم جمع السائل المنوي باستخدام 10 كباش عواسية بالغة كل أسبوعين باستخدام جهاز القذف الكهربائي (قذفة واحدة/ كبش). تم تقدير الخصائص الفيزيائية للسائل المنوي وتركيز إنزيمات AST و ALT و ALP و LDH و ACP والعناصر المعدنية (الصوديوم، البوتاسيوم، الكالسيوم، الزنك، الكلور، الفسفور، المغنسيوم، الحديد) في السائل المنوي. أظهرت النتائج وجود علاقة ارتباط معنوية بين إنزيم AST ($r = -0.671$)، ($r = -0.500$)، ($r = -0.587$)، ($r = 0.587$) مع الاس الهيدروجيني والحركة الفردية والنطف الحية والميتة. كما لوحظ وجود علاقة ارتباط معنوية بين إنزيم ALT ($r = -0.578$) و ($r = -0.560$) و ($r = 0.560$) مع الاس الهيدروجيني والنطف الحية والميتة على التوالي. تم الحصول على علاقة سلبية بين ALP مع حجم السائل المنوي ودرجة الحموضة والكتلة والحركة الفردية والحيوانات المنوية الحية وسلامة غشاء النطف، في حين كانت العلاقة إيجابية مع تركيز النطف، والنطف الميتة والمشوهة. أظهر إنزيم LDH علاقة إيجابية مع حجم السائل المنوي والجماعية والفردية والنطف الحية والمشوهة، في حين وجدت علاقة سلبية مع الاس الهيدروجيني، تركيز النطف، والنطف الميتة وسلامة غشاء النطف. كان لإنزيم ACP علاقة إيجابية مع حجم السائل المنوي والحركة الجماعية والفردية، تركيز النطف والنطف الميتة وسلامة غشاء الخلية. كان لعنصري الصوديوم والكلور علاقة ارتباط معنوية مع حجم السائل المنوي، وعنصر الكلور مع الحركة الجماعية. كما ان عناصر الزنك والكالسيوم والمغنسيوم والحديد أظهرت علاقة إيجابية معنوية مع تركيز النطف، في حين كان عنصر الفسفور ذات علاقة ارتباط معنوية سلبية معها. وعلاقة معنوية إيجابية بين الكالسيوم مع الحركة الفردية النطف الحية وتركيز النطف، في حين كانت العلاقة معنوية سلبية مع النطف الميتة والمشوهة. لذلك، فإن طبيعة الإنزيمات والعناصر الكيميائية للسائل المنوي هي مؤشر أساسي في تقييم جودة السائل المنوي للكباش العواسية.

الكلمات المفتاحية: الاغنام، العناصر، الانزيمات، السائل المنوي.

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