SOME BIOCHEMICAL ATTRIBUTES IN SEMINAL PLASMA OF IRAQI BUFFALO BULLS AND THEIR RELATION TO THE SEMEN QUALITY

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ABSTRACT

This study was undertaken to figure out the relationship between some biochemical characteristics in seminal plasma with the semen quality of Iraqi buffalo bulls (*Bubalus bubalis*). Semen was diluted with the Tris extender and divided based on the sperm cell's individual motility into two groups. The first group included good-semen quality (GS; n=3), whereas, the second group included poor-semen quality (PS; n=5). The differences between GS and BS groups in all seminal biochemical attributes lacked significance. A negative and significant (P≤0.05) correlation coefficient was noticed between cholesterol concentration and both principal and terminal sperms abnormality (r= -0.52), and total abnormalities (r= -0.53). A positive and significant (P≤0.05) correlation coefficient was shown between cholesterol concentration and normal sperm percentage (r= 0.53). Moreover, there was a negative and significant correlation (P≤0.05) between the triglyceride concentration and each of the percentages of individual sperm motility (r = -0.50) and of live sperm (r = -0.51). It can be concluded that some biochemical characteristics of seminal plasma can be used to predict the semen quality and fertility of Iraqi buffalo bulls.

Keywords: cholesterol, triglycerides, semen, fertility prediction, Buffalo.

بعض الصفات الكيمياحيوية في البلازما المنوية لثيران الجاموس العراقي وعلاقتها بنوعية السائل المنوي

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المستخلص

أجريت هذه الدراسة بهدف الكشف عن علاقة بعض الصفات الكيمياحيوية في البلازما المنوية بنوعية السائل المنوي لثيران الجاموس العراقي (*Bubalus bubalis*). تم تخفيف السائل المنوي باستخدام مخفف Tris وقسم حسب النسبة المئوية للحركة الفردية لثيران الجاموس الى مجموعتين، ضمت المجموعة الاولى سائل منوي جيد النوعية (GS،3=n)، في حين ضمت المجموعة الثانية سائل منوي رديء النوعية (PS،5=n). اظهرت النتائج انعدام الفروق المعنوية بين مجموعتي السائل المنوي الطازج الجيد والرديء النوعية في جميع الصفات الكيمياحيوية في البلازما المنوية. كانت هنالك علاقة ارتباط سالبة ومعنوية (P≤0.05) بين تركيز الكولسترول والنسبة المئوية لتشوهات القطعة الرئيسية والنهائية لذيل النطف (r=−0.52) والتشوهات الكلية للنطف (r=−0.53)، في حين كان الارتباط موجب ومعنوي(P≤0.05) بين تركيز الكولسترول والنسبة المئوية للنطف الطبيعية (r=0.53). من جانب أخر، كانت هنالك علاقة الارتباط سالبة ومعنوية (P≤0.05) بين تركيز الكلسريدات الثلاثية وكل من النسبة المئوية لحركة النطف الفردية (r=−0.50) والنسبة المئوية للنطف الحية (r=−0.51). يمكن الاستنتاج بإمكانية استعمال بعض الصفات الكيمياحيوية للبلازما المنوية للتنبؤ بنوعية السائل المنوي وخصوبة ثيران الجاموس العراقي.

الكلمات المفتاحية: الكولسترول، الكلسريدات الثلاثية، السائل المنوي، التنبؤ بالخصوبة، الجاموس.

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INTRODUCTIO

Biomarkers, such as various proteins and other biochemical attributes, lead an essential role in the biological systems of males and females (5, 6, 10, 22, 23, 24, 34), but the detailed information for its presence in seminal plasma, importance in sperm metabolism, and their relation with bull fertility is still unknown (18,21,25,39,41). It is well-known that buffalo semen is characterized by high contents of fructose, acid and alkaline phosphatase, and inorganic phosphorus but lower content of antioxidants compared to bull semen (8,9,13,15,40,43). Moreover, lower activities of amylase (40), acetylcholine esterase, and lesser cholesterol concentration (25, 31) were also observed compared to the bull semen. On the other hand, Ezz et al. (25) showed that the plasma membrane of buffalo bull sperm is rich in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities that increase in seminal plasma during freezing-thawing processes due to the cholesterol loss from sperm membrane, and this is associated with the plasma membrane lysis. The presence of plasma membrane enzymes indicates membrane stability. So, it is expected that the freezing-thawing process of buffalo bull semen will increase the enzyme's activity in seminal plasma compared to fresh semen. The alkaline phosphatase (ALP) enzyme has a sperm vitality negative effect during the cryopreservation period. Increasing the enzyme leads to a sperm's ability to decrease oxidative stress resistance, and thus sperm viability weakening. Therefore, the ALP activity increase is associated with the decreasing percentages of sperm individual motility and livability, concomitantly with reducing dehydrogenase activity and thus reducing the fructose uptake by the buffalo bull's sperm (36). Furthermore, the seminal plasma of the buffalo bulls has little protein and cholesterol and more triglyceride concentrations. The sperms have more fatty acid concentration than the Holstein bulls. Further, the sphingolipids are potential markers of sperm cryotolerance resistance for Mediterranean buffalo bulls (33). With the establishment of the artificial insemination project for buffaloes in Iraq six years ago, the actual need began to detect the biomarkers in the seminal plasma of Iraqi buffalo bulls as a tool for predicting semen quality and fertility at an early age. These will contribute to developing plans for increasing the conception and pregnancy rates for the buffalo inseminated by these bulls. In a previous study (7, 8, 9), encouraging results were obtained by detecting some amino, fatty, and organic acids small amounts of antioxidants in seminal plasma and their relationship to the semen quality of Iraqi buffalo bulls. The lack of a previous study concerning the detection of some biochemical attributes in the seminal plasma of Iraqi buffalo bulls and their relation to the quality of fresh and cryopreserved semen prompted us to conduct this attempt.

MATERIALS AND METHODS

Animals and semen collection

The study was undertaken at the Artificial Insemination Department (AID) belonging to the Directorate of Animal Resource, Ministry of Agriculture, Iraq, from December 2021 to October 2022. Eight Iraqi buffalo bulls (*Bubalus bubalis*) trained for semen collection using the artificial vagina, with ages ranging between 5.5-6 years and a body weight ranging between 500-700 kg/bull. All bulls were in good health and under continuous veterinary supervision. All animals fed 5 kg of concentrate diet/animal/day consisted of 35% barley, 33% wheat bran, 10% yellow corn, 20% soybean meal, 0.5% limestone, and 1% vitamins and minerals. The crude protein percentage and total energy amount of the diet were 18% and 2164 kilocalories/kg, respectively. Good fresh semen (sperm cell individual motility percentage is ≥ 50%) was collected from three buffalo bulls, whereas poor fresh semen (sperm cell individual motility percentage is < 50%) was collected from five buffalo bulls using artificial vaginas as once ejaculate / week/ bull for eight weeks.

Semen processing and evaluation

The ejaculate volume was measured in a graduated tube, and the sperm concentration was calculated using a spectrometer (Hamilton- Technologies IMV, France). The fresh semen characteristics have evaluated during 96 hr., three, and six months, post-cryopreservation (PC). The percentages of sperm's motility, livability, and abnormality are assessed based on Srivastava and Pande

(36). The integrity percentages of sperm plasma membrane and acrosome were examined using the method of Eidan (19). One milliliter of each ejaculate from either GS or PS groups was taken, pooled, and centrifuged (3000 rpm for 15 minutes) for harvesting the seminal plasma. The ALP, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities of Glucose, cholesterol, HDL, LDL, and vLDL concentrations were evaluated (1, 2, 36).

Statistical analysis

The statistical computation was done using SAS program based on the complete randomized design (CRD) to investigate the relationship between the biochemical traits in seminal plasma and the semen quality of the buffalo bulls. Means with significant differences were compared using the Duncan multiple range test. Two statistical models were used as follows:

The first model explained the effect of semen quality (Good or poor).

$Y_{ij}= \mu+ F_i+ e_{ij}$

Y_{ij}=dependent variables (semen characteristics).

μ= overall mean

F_i= effect of semen quality (good or poor)

$e_{ij=}$ error term

The second model explained the preservation periods' effect on semen characteristics within each group.

$Y_{ij} = \mu + Pi + eij$

Y_{ij} = dependent variables (Semen characteristics).

μ= overall mean

Pi= effect of the period (cooling 5˚ C, 96 hours, 3rd and 6th months PC).

$e_{ij=}$ error term.

RESULTS AND DISCUSSION

Biochemical characteristics in seminal plasma: The differences between the GS and PS groups of fresh semen quality in all biochemical attribute concentrations in the seminal plasma lacked significance (Table 1). However, the numerically increasing percentages of these traits in the GS group compared to the PS group (Table 1).

Table1. Some biochemical characteristics of seminal plasma for good and poor-quality semen of Iraqi buffalo bulls based on individual motility percentage (Mean SE).

Biochemical characteristics in seminal plasma	Good-quality semen (n=3)	Poor-quality semen (n=5)	Increasing percentage	Level of significance
Glucose (mg /dl)	540.01 ± 217.47	576.29 ± 138.15	6.72	NS
Cholesterol (mg /dl)	177.9 ± 56.53	126.23 ± 43.17	40.93	NS
Triglycerides (mg /dl)	680.55 ± 229.53	679.07 ± 151.60	0.22	NS
High-density lipoprotein (mg /dl)	2.51 ± 0.27	2.32 ± 0.42	8.18	NS
Low-density lipoprotein (mg /dl)	32.56 ± 13.97	37.27 ± 13.70	14.46	NS
Very low-density lipoproteins (mg /dl)	133.28 ± 47.42	119.83 ± 33.88	11.22	NS
Aspartate aminotransferase (U/L)	79.94 ± 26.67	43.58 ± 12.037	83.43	NS
Alanine aminotransferase (U/L)	112 ± 24.34	152.81 ± 40.42	36.44	NS
Alkaline Phosphatase (U/L)	1149.67 ±528.45	899.3 ± 212.48	27.84	NS

NS: Non-significant

Characteristics of good and poor-fresh semen quality: Non-significant differences have been observed between GS and PS fresh groups in ejaculate volume and sperm concentration (Table 2). On the other hand, higher (P≤ 0.0001) percentages of mass activity, sperm individual motility, livability, plasma membrane integrity, acrosome integrity, and normal sperm have been noticed for GS than for PS groups (Table 2). Lesser abnormality percentages of sperm head ($P \leq 0.04$), tail principal and terminal ($P \leq 0.002$), and total sperms ($P \leq 0.0001$) for GS than for PS groups, whereas the differences between the two groups in sperm's tail midpiece abnormalities lacked significance (Table 2).

Table 2. Comparison of fresh good and poor-semen characteristics of buffalo bulls using microscopic examination method (Mean ± SE).

Semen characteristics	Good-quality semen (n=3)	Poor-quality semen (n=5)	Level of significance
Sperm individual motility (%)	33.05 ± 3.00 A	18.16 ± 1.38 B	P≤0.0001
Live sperms (%)	48.72 ± 2.79 A	34.7 ± 1.77 B	P≤0.0001
Sperm's head abnormalities (%)	4.22 ± 0.34 B	5.66 ± 0.24 A	P≤0.001
Sperm's tail midpiece abnormalities (%)	0.83 ± 0.12 A	1.1 ± 0.1 A	NS
Sperm tail's principal and terminal abnormalities (%)	6.66 ± 0.41 B	*9.0 ± 0.37* A	P≤0.0002
Total sperm abnormalities (%)	11.66 ± 0.71 B	15.76 ± 0.53 A	P≤0.0002
Sperm's plasma membrane integrity (%)	49.77 ± 2.35 A	33.9 ± 1.54 B	P≤0.0001
Sperm's acrosome integrity (%)	52.61 ± 2.20 A	36.93 ± 1.749 B	P≤0.0001
Normal sperm (%)	88.33 ± 0.71 A	84.23 ± 0.53 B	P≤0.0001

Means with different superscripts within each row indicate significant differences between good and poor-quality semen groups. NS: Non-significant

Characteristics of good and poor cryopreserved semen quality: The GS group exhibited a higher (P≤ 0.0001) percentage of sperm individual motility (35.27 ± 2.60%) and live sperm (56.05±2.36%) than PS groups 96 hr PC (Table 3). Lesser (P≤0.0002) abnormality percentages of sperm head, sperm tail principal and terminal, and total sperms were recorded for GS than for PS groups (Table 3). Moreover, a lesser P≤0.01) percentage of sperm tail midpiece abnormalities was noticed in GS than in PS groups (Table 3). On the other hand, the GS group recorded higher (P≤ 0.0001) percentages of plasma membrane integrity, acrosome integrity, and normal sperm than the PS group at 96 hr. PC (Table 3). Higher (P≤0.0001) percentages of sperm individual motility (33.05 ± 3.00 vs. 18.16 ± 1.38 %) and live sperm (48.72 ± 2.79 vs. 34.7 ± 1.77 %) were observed for the GS than for the PS groups three months PC (Table 4). On the other hand, lesser (P≤0.001) abnormality percentages of sperm heads (4.22 ± 0.34 vs. 5.66 ± 0.24 %) were shown for the GS than for the PS groups within a similar preservation period (Table 4). Non-significant differences were noticed between the GS and the PS groups in the abnormality percentages of sperm's tail midpiece, whereas lesser (P≤0.0002) abnormality percentages in sperm's tail principal and terminal and total sperms were observed for the GS than for the PS groups three months PC (Table 4). The GS group exhibited higher (P≤0.0001) percentages of plasma membrane integrity, acrosome integrity, and normal sperms than the PS group three months PC (Table 4). A conspicuous increase (P≤0.0001) in percentages of the sperm individual motility (30.83 ± 2.68 vs. 15.16 ± 1.05 %) and live sperm (44.11 ± 2.64 vs. 30.5 ± 1.58%) was shown for the GS than for the PS groups six months PC (Table 5). On the other hand, a pronounced decrease (P≤0.0004 or P≤0.001) in the abnormality percentages of sperm's head, sperm tail's principal and terminal and total sperms were observed for the GS group compared to the PS group within a similar preservation period (Table 5).

Table 3. Comparison of good and poor-quality semen characteristics of Iraqi buffalo bulls 96 hr. post-cryopreservation using microscopic examination method (Mean ± SE).

Semen characteristics	Good-quality semen (n=3)	Poor-quality semen (n=5)	Level of significance
Sperm individual motility (%)	35.27 ± 2.60 A	19.66 ± 1.37 B	P≤0.0001
Live sperms (%)	56.05 ± 2.36 A	40.4 ± 1.72 B	P≤0.0001
Sperm's head abnormalities (%)	4.55 ± 0.34 B	6.1 ± 0.21 A	P≤0.000*2*
Sperm's tail midpiece abnormalities (%)	0.72 ± 0.15 B	1.13 ± 0.07 A	P≤0.01
Sperm tail's principal and terminal abnormalities (%)	6.77 ± 0.43 B	9.16 ± 0.27 A	P≤0.000*2*
Total sperm abnormalities (%)	12.05 ± 0.70 B	16.43 ± 0.42 A	P≤0.000*2*
Sperm's plasma membrane integrity (%)	54.61 ± 2.11 A	38.66 ± 1.79 B	P≤0.0001
Sperm's acrosome integrity (%)	56.77 ± 2.09 A	41.86 ± 1.88 B	P≤0.0001
Normal sperm (%)	87.94 ± 0.70 A	83.56 ± 0.42 B	P≤0.0001

Means with different superscripts within each row indicate significant differences between good and poor-quality semen groups.

Table 4. Comparison of good and poor-quality semen characteristics of Iraqi buffalo bulls three months post-cryopreservation using microscopic examination method (Mean ± SE).

Semen characteristics	Good-quality semen (n=3)	Poor-quality semen (n=5)	Level of significance
Sperm individual motility (%)	30.83 ± 2.68 A	15.16 ± 1.05 B	P≤0.0001
Live sperms (%)	44.11 ± 2.64 A	30.5 ± 1.58 B	P≤0.0001
Sperm's head abnormalities (%)	4.22 ± 0.38 B	6 ± 0.27 A	P≤0.0004
Sperm's tail midpiece abnormalities (%)	0.83 ± 0.12 A	1.13 ± 0.10 A	NS
Sperm tail's principal and terminal abnormalities (%)	6.94 ± 0.51 B	9.16 ± 0.40 A	P≤0.001
Total sperm abnormalities (%)	12 ± 0.86 B	16.3 ± 0.62 A	P≤0.0004
Sperm's plasma membrane integrity (%)	44.33 ± 2.30 A	30.03 ± 1.55 B	P≤0.0001
Sperm's acrosome integrity (%)	47.22 ± 2.25 A	33.36 ± 1.57 B	P≤0.0001
Normal sperm (%)	88 ± 0.86 A	83.7 ± 0.62 B	P≤0.0001

Means with different superscripts within each row indicate significant differences between good and poor-quality semen groups. NS: Non-significant

Whereas the differences between the two groups in the abnormality percentages of sperm tail midpiece lacked significance at six months PC (Table 5). Moreover, the GS group exhibited higher (P≤ 0.0006) values of plasma membrane integrity (44.3 3 ± 2.30 vs. 30.03 ± 1.55%), acrosome integrity (47.22 ± 2.25 vs. 33.36 ± 1.57%), and normal sperms than the PS group six months PC (Table 5).

Table 5. Comparison of good and poor-quality semen characteristics of Iraqi buffalo bulls six months' post-cryopreservation using microscopic examination method (Mean ± SE).

Semen characteristics	Good-quality semen (n=3)	Poor-quality semen (n=5)	Level of significance
Sperm individual motility (%)	30.83 ± 2.68 A	15.16 ± 1.05 B	P≤0.0001
Live sperms (%)	44.11 ± 2.64 A	30.5 ± 1.58 B	P≤0.0001
Sperm's head abnormalities (%)	4.22 ± 0.38 B	6 ± 0.27 A	P≤0.0004
Sperm's tail midpiece abnormalities (%)	0.83 ± 0.12 A	1.13 ± 0.10 A	NS
Sperm tail's principal and terminal abnormalities (%)	6.94 ± 0.51 B	9.16 ± 0.40 A	P≤0.001
Total sperm abnormalities (%)	12 ± 0.86 B	16.3 ± 0.62 A	P≤0.0004
Sperm's plasma membrane integrity (%)	44.33 ± 2.30 A	30.03 ± 1.55 B	P≤0.0001
Sperm's acrosome integrity (%)	47.22 ± 2.25 A	33.36 ± 1.57 B	P≤0.0001
Normal sperm (%)	88 ± 0.86 A	83.7 ± 0.62 B	P≤0.0001

Means with different superscripts within each row indicate significant differences between good and poor-quality semen groups. NS: Non-significant

The correlation coefficient between semen characteristics and biochemical traits in seminal plasma: A negative and significant (P≤0.05) correlation coefficient was noticed between glucose concentration and both percentages of sperm individual motility (r=-0.59) and live sperms (r=-0.51; Table 6). On the other hand, the correlation coefficient between cholesterol concentration and abnormality percentages of sperm tail principal and terminal (r = -0.52) and total sperms (r = -0.53) was negative and significant (P ≤ 0.05; Table 6). A conspicuous (P≤0.05) and positive correlation coefficient was noticed between cholesterol concentration and normal-sperms percentage (r=0.53; Table 6). Moreover, a negative and significant (P≤0.05) correlation coefficient was observed between triglyceride concentration and both the percentage of sperm individual motility (r = -0.50) and live sperms (r =-0.51; Table 6). The current results revealed a negative and significant (P≤0.05) correlation coefficient between LDL concentration in the seminal plasma and the percentage of sperm individual motility (r = -0.52). Similarly, a negative and significant (P≤0.05) correlation coefficient was found between vLDL concentration and percentages of sperm individual motility (r =-0.52) and live sperms (r = -0.51; Table 6).

Table 6. The correlation coefficient between some semen characteristics of Iraqi buffalo bulls and biochemical characteristics in seminal plasma

Biochemical / Semen Characteristics	Glucose (mg /dl)	Cholesterol (mg /dl)	Triglyceride (mg /dl)	High-density lipoprotein (mg /dl)	Low-density lipoprotein (mg /dl)	Very low-density lipoproteins (mg /dl)	Aspartate aminotransferase (U/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)
Ejaculate volume (ml)	0.33- NS	0.23 NS	0.41- NS	0.14- NS	0.02- NS	0.30- NS	0.30- NS	0.01- NS	0.37- NS
Sperm concentration (million/ml)	0.08 NS	0.15 NS	0.09 NS	0.32 NS	0.05¯ NS	0.08 NS	0.20 NS	0.31 NS	0.06 NS
Mass activity (%)	0.21- NS	0.01- NS	0.11- NS	0.22 NS	0.24- NS	0.11- NS	0.53 *	0.33 NS	0.02- NS
Individual motility (%)	0.59- *	0.41- NS	0.50- *	0.36- NS	0.52- *	0.52- *	0.51- *	0.36 NS	0.26- NS
Live sperms (%)	- 0.51 *	0.21- NS	0.51- *	0.29- NS	0.29- NS	0.51- *	0.31 NS	0.08 NS	0.08- NS
Head abnormalities (%)	0.30- NS	0.38- NS	0.31- NS	0.10 NS	0.18- NS	0.30- NS	0.03- NS	0.43 NS	0.43- NS
Midpiece abnormalities (%)	0.05- NS	0.06 NS	0.02- NS	0.25 NS	0.13- NS	0.02- NS	0.27 NS	0.37 NS	0.02 NS
Principal & terminal abnormalities (%)	0.25- NS	0.52- *	0.28- NS	0.15- NS	0.07- NS	0.29- NS	0.40- NS	0.18 NS	0.32- NS
Total abnormalities (%)	0.31- NS	0.53- *	0.33- NS	0.04- NS	0.14- NS	0.34- NS	0.27- NS	0.33 NS	0.50- *
plasma membrane integrity (%)	0.05- NS	0.17 NS	0.02- NS	0.05- NS	0.01 NS	0.02 NS	0.36 NS	0.08- NS	0.28 NS
Acrosome integrity (%)	0.06- NS	0.18 NS	0.03- NS	0.03- NS	0.03- NS	0.02 NS	0.41 NS	0.12- NS	0.23 NS
Normal sperm (%)	0.31 NS	0.53 *	0.33 NS	0.04 NS	0.14 NS	0.34 NS	0.27 NS	0.33- NS	0.52 *

NS: Non-significant. *: P≤0.05

Furthermore, a positive and significant (P≤0.05) correlation coefficient was observed between AST activity in the seminal plasma and percentage of mass activity (r =0.53), whereas the correlation coefficient between AST activity and sperm individual motility percentage was negative and significant (r =-0.51; P≤0.05; Table 6). Concomitantly, a negative and significant (P≤0.05) correlation coefficient was noticed between ALP activity in the seminal plasma and the percentage of total abnormality (r =-0.50), whereas the correlation coefficient between ALP activity and the normal sperm percentage (r = 0.52) was positive and significant (P≤0.05; Table 6). The non-significant differences between GS and PS bulls of glucose concentrations in seminal plasma may be due to seminal vesicles' ability of both groups to secrete moderate amounts of glucose into the seminal plasma and use it for sperm energy production (36). Although fructose is the principal sugar secreted by seminal vesicles and is necessary for sperm metabolism and motility, sperm consume about half the glucose quantity needed for energy production and motility (36). It is worth mentioning that fructose can replace glucose to give sufficient amounts of energy for sperm motility. Sperm also use glucose as an energy source even if fructose concentration is 50 folds higher than glucose because it is the principal metabolite of seminal vesicles, which turns into fructose used as a sperm energy source in semen (36). The current results agreed with those reported by Almadaly et al. (11), who did not find significant differences in the glucose concentration of seminal plasma between high and low-fertility bulls. Zhu et al. (44) found that lowering glucose concentration pre-cryopreservation led to increasing sperm motility by activating the mitochondria for post-thaw ATP production. They noticed that cooling sperms pre-cryopreservation hexokinase activity, LDH, and lactate concentrations in high-content compared with low-content glucose treatments. Lowering glucose levels helped in the inactive spermatozoa lysis during cryopreservation and may contribute to improving the conception rate of the buffaloes by using post-thawed sperm for insemination protocols. Therefore, a high glucose level in seminal plasma may reduce the sperm individual motility of Iraqi buffalo bulls, 96 hr, three and six PC. The significant and negative correlation coefficient between the sperm individual motility and glucose concentration (r =-0.50) in the current results (Table 1) may support the existing notion. The lack of significant differences between GS and PS groups in AST and ALP activity in seminal plasma indicates a lower percentage of abnormal or dead sperms within these two groups. A higher percent of dead and abnormal sperms contributed to the exit of these enzymes into the seminal plasma as a result of sperm membranes rupture being mainly intracellular enzymes that are secreted in valuable quantities by the accessory sex glands such as seminal vesicles and prostate, testicles, and epididymis to a lesser extent, lead to increase the sperm metabolic rate (14). The lower percentages of the sperm head, tail principal, and terminal, and total abnormalities for both GS and PS groups to less than 20%, and the utmost increase of the normal sperm percentage over 70% 96 hr, three and six months PC (Tables 3-5) greatly supports this hypothesis. Thus, the activity estimation of AST, ALT, and ALP enzymes in the seminal plasma reflects the functional state of accessory sex glands, sperm metabolic activity, and vitality. The numerical and non-significant increase of cholesterol concentration in the seminal plasma for GS than PS groups may be one of the main reasons behind the superiority in the percentage of sperms' motility, livability, plasma membrane integrity, acrosome integrity, normal sperm percentage, and a decrease in total sperm abnormalities (Table 3-5). It is worth mentioning that cholesterol plays a pivotal role in sperm motility, capacitation, acrosomal reaction, and fertility (37). The positive and significant correlation coefficient between the cholesterol concentration and the percentages of normal sperm (r = 0.53), plasma membrane integrity (r = 0.17), acrosome integrity (r = 0.18), ejaculate volume (r = 0.23) and sperm concentration (r = 0.15) in the current results (Table 6), indicate a cholesterol role in the buffalo bull's fertility. The present results did not agree with those reported by El-Sayed et al

(24), who found a positive and significant correlation coefficient between cholesterol concentration in the seminal plasma and some semen characteristics of Egyptian buffalo bulls (*Bubalus bubalis*). On the other hand, the current results agreed with those reported by Almadaly et al (11), who did not find significant differences in the cholesterol concentration in seminal plasma of the high and low-fertility bulls. The numerical non-significant increase of the triglycerides concentration in the seminal plasma of the GS group (0.22%) compared to the PS group (Table 1) may be due to changes in its concentration between high-fertile and low-fertile buffalo bulls. Triglycerides are one of the principal energy substances available for sperm metabolism, and therefore, triglycerides' low levels may lead to insufficient energy and reduced sperm motility and fertility. Accordingly, Khoshvaght et al (30) found that adding dietary fish oil containing omega-3 and omega-6 led to a quality improvement in fresh and cryopreserved semen of Holstein bulls. The current results agreed with Almadaly et al. (11) data, who did not find significant differences in the LDL concentration in seminal plasma for the high and low-fertility bull's semen. Decreasing the protein content in the seminal plasma reduces the ability of the sperm to be preserved for long periods, thus reducing the quality and fertility of sperm (17). Lipoproteins play a crucial role in the structure and function of the sperm plasma membrane. However, there is evidence that ejaculate volume, sperm motility, and concentration are affected by lipid types in the seminal plasma (32). Some types of seminal plasma proteins are closely related to the fertility of bulls (16, 28, 33, 35). It is worth noting that the current study achieved higher percentages of plasma membrane integrity (71.55-82%) and acrosome integrity (72.65-84.11%) for good and bad quality semen (Table 2) compared to what was obtained by Chavda et al (16) in Indian Jaffarabadi buffalo bulls (45.54 ± 0.70 and 51.08 ± 0.72% for plasma membrane and acrosome integrity PC, respectively. On the other hand, these percentages were similar to those reported by Sultan and Eidan (38), who obtained pronounced data on plasma membrane integrity (72.61 - 74.23%) and acrosome integrity (76.03 - 76.25%) of fresh Holstein bull semen. Further, sperm plasma membrane integrity is a prerequisite for good sperm motility and a high fertilization rate, and semen samples with low sperm progressive motility often suffer from a defect in the plasma membrane (4, 27). The percentage results of live sperm three months PC in the current study (48.72 ± 2.79; Table 4) were higher than what was obtained by Al-Nuaimi et al. (12; 44.28 ± 3.62%) and less than those obtained by Eidan (19; 53.71 ± 2.08%) and Abdulkareem and Al-Zaidi (3; 54.66 ± 2.49%). In contrast, the percentage of live sperm after six months of cryopreservation (44.11± 2.64; Table 5) was higher than what was obtained by Kadoom et al (29; 31.1 ± 1.24%) in buffalo bulls and lower than what was obtained by Hassan and Eidan (26) ; 79.64 ± 0.90%) in Holstein bulls 96 hr PC.

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