METABOLITES AND SEMEN CHARACTERISTICS IN DIFFERENT BULLS FERTILITY

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ABSTRACT

This study was investigated the relationship between Holstein bulls' fertility with some biomarkers (amino، fatty، and organic acids) and semen characteristics. The study included exploring the relationship among the fertility of Holstein bulls، several biomarkers، and semen characteristics in straws (20-25 straws/bull) preserved for three years. The cows were artificially inseminated (9-10 cows/bull) from semen. Based on the fertility rate، the bulls were divided into three groups، the first bulls of medium fertility (<65%)، the second of good fertility (65-79%)، and the third of high fertility (>80%). The semen characteristics were evaluated، and the concentration of some amino acids and organic fatty acids was measured in the seminal plasma of bulls using gas chromatography and HPLC. The results revealed significant differences in the fertility rate among the different groups of bulls in favor of the highly fertile bulls. The results did not show any differences in most of the characteristics of the semen، except for a significant increase in the percentage of live sperm in good bulls compared to highly fertile ones. There were no significant differences for all the concentrations of carboxylic and most amino and fatty acids concentrations، except for an increase in the concentration of glycine acid (P<0.05) and a decrease in the concentration of omega 9 (P<0.05) in the highly fertile bulls compared to the medium fertile ones. In conclusion، glycine and omega-9 in the seminal plasma of bulls can be regarded as biomarkers of their fertility and the level of carboxylic acids (volatile fatty acids) can be adopted as an indicator of semen quality or energy level in the diet

Keywords: Conception rate، sperm، seminal plasma attributes، amino acid، carboxylic acids.

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المواد المتأيضة وصفات السائل المنوي لدى ثيران مختلفة الخصوبة.

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المستخلص

أجريت هذه الدراسة بهدف بيان العلاقة بين خصوبة ثيران الهولشتاين مع بعض المؤشرات الحيوية (الاحماض الامينية والدهنية والعضوية) وصفات السائل المنوي. تضمنت الدراسة دراسة العلاقة بين خصوبة ثيران الهولشتاين وعدد من المؤشرات الحيوية كدلائل ايضية وصفات السائل المنوي في القصبات المحفوظة بالتجميد (20–25 قصبة / ثور) لمدة ثلاث سنوات. تم تلقيح الابقار اصطناعيا (9–10 بقرة/ ثور) من السائل المنوي وعلى اساس نسبة الخصوبة قسمت الثيران الى ثلاثة مجاميع، الاولى ثيران متوسطة الخصوبة (اقل من 65 %) والثانية جيدة الخصوبة (65–79%) والثالثة عالية الخصوبة (اعلى من 80 %). تم تقييم صفات السائل المنوي، كما تم في الوقت نفسه قياس تركيز بعض الأحماض الامينية والدهنية العضوية في البلازما المنوية للثيران بطريقة Gas chromatography وHPLC. أظهرت النتائج وجود فروق معنوية في نسبة الخصوبة بين مجاميع الثيران المختلفة لصالح الثيران العالية الخصوبة. ولم تظهر النتائج وجود فروق في معظم صفات السائل المنوي باستثناء زيادة النسبة المئوية للنطف الحية معنويا لدى الثيران الجيدة مقارنة بالعالية الخصوبة. ولم يكن هنالك فروق معنوية لجميع تراكيز الأحماض العضوية ومعظم تراكيز الأحماض الامينية والدهنية باستثناء زيادة تركيز حامض الكلايسين (P<0.05) وانخفاض تركيز الأوميكا 9 (P<0.05) لدى الثيران العالية الخصوبة مقارنة مع المتوسطة الخصوبة. يمكن الاستنتاج بإمكانية اعتماد الحامض الاميني الكلايسين والاوميغا 9 في البلازما المنوية للثيران كدلائل ايضية لخصوبتها كما يمكن اعتماد مستوى الاحماض الكاربوكسيلية (الاحماض الدهنية الطيارة) كمؤشر لنوعية السائل المنوي او مستوى الطاقة في العليقة.

الكلمات المفتاحية: نسبة الحمل، احماض امينية واحماض كاربوكسيلية، نطف وصفات البلازما المنوية.

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INTRODUCTION

The bull's fertility is critical in cows' artificial or natural insemination. Its economic importance within sustainable development systems (12، 18، 26، 27، 45). The bull's fertility is related to producing sperm and fertilizing the egg. Artificial insemination (AI) technology has become principal and commercially available for cow breeders through its efficient use of genetically superior bulls، genetic factors improvement، and productive cows' characteristics. The success of AI technology depends on the quality of fresh semen and the ability to dilute and maintain its good quality post-cryopreservation and thawing. (2،20، 24 32،،33،35). Moreover، success also depends on the sperm freeze ability and enhancing the cow's pregnancy rate consequently (20، 21، 24، 29). Male and female fertility is affected by many factors like management، feeding، season، disease، stress، age، genetics، and hormones (7، 8، 9، 22، 23، 43، 44). The use of sperms originating from low-fertility bulls caused a great economic loss (36). So، the main objective of semen evaluation is to predict the bull's fertile ability by finding correlation coefficients between semen quality and fertility (18،36). The classical methods of evaluating semen for predicting bull fertility are still inaccurate. Some bulls produce high-quality sperm but are infertile. Genes، reproduction factors، proteins، and metabolites are factors that influence bull fertility (18،23،35،45،46). The seminal plasma contains proteins، steroid hormones، ions، and metabolites. Amino، fatty، carboxylic acids، monosaccharides، nucleotides، and minerals are regarded as metabolites (6، 15، 21). Metabolites are the end products of metabolic pathways. Metabolites play a crucial role in regulating sperm metabolic activities and their physiology. Fatty acids provide energy and maintain sperm membrane integrity، fluidity، and permeability under freezing conditions. Fatty acids are associated with the membrane's phospholipid bilayer (32). Sperm can benefit from the fatty acids in the surrounding media to maintain integrity، viability، and membrane effectiveness. Seminal plasma fatty acids predict to cryopreserve ability in males and bulls (21، 34). Semen amino acids have antioxidant properties and protect against sperm freezing. Recently، 63 metabolized compounds، including 21 amino acids، were identified in the seminal plasma of high- and low-fertility bulls (48). Ugur et al. (47) and Abdulkareem et al. (6) indicated that there is a relationship between the concentration of amino acids in seminal plasma and the different sperm-freezing abilities of Holstein bulls. The semen carboxylic acids are a source of sperm energy. Menezes et al. (37) showed small quantities of acetic، benzoic، carbonate، and 2-keto butyric carboxylic acids in the bull semen، but Kumar et al. (30) reported that citrate has a high relationship with increasing the fertility of bulls. Velho et al. (48) showed that lactic and citric acids are among the five most abundant metabolites in the seminal plasma of bulls. Citric acid is a bull fertility indicator because of its effect on transport، acrosomal reactions، and fertilization. Also، it serves as an energy source and pH regulator by binding or chelating calcium، zinc، and magnesium ions. Meneze et al. (37) found that the percentage of carboxylic acids was 9.1% of the total metabolites. Klein et al. (28) showed that the seminal plasma of bulls contained 17% carboxylic acids. Because of their positive effects on sperm cryopreservation resistance، measuring metabolic markers in seminal plasma has become a quick guide for identifying fertile or infertile males (6،21). Previous studies did not investigate the relationship of these biomarkers with different types of bull fertility. Limited studies investigated the relationship of these markers with high and low-bulls fertility without addressing medium and good fertility. Therefore، this experiment was conducted to study the relationship of several fatty، amino، and carboxylic acids as metabolite markers and post-cryopreservation semen characteristics in Holstein bulls' seminal plasma with high، good، and medium fertility percentages.

MATERIALS AND METHODS

Experimental design

The study was carried out at the Artificial Insemination Department belonging to the Directorate of Animal Resource، Ministry of Agriculture. Twenty-one Holstein bulls of 2-7 years old and 538-750 kg body weight were

used currently. All Holstein bulls were healthy، disease-free، and under constant veterinary supervision. All bulls were allocated on a standardized diet، as a concentrate ration (protein18% and 2146 kcal) was provided daily at a rate of 4-6 kg/bull. Roughage consisted of alfalfa hay (7-9 kg/bull/day) besides green forage (50-60 kg/bull/day). Fresh water and salt blocks were available ad libitum to the bulls. Ten multiparous crossbred Holstein cows (with body condition scores between 2.75 and 3) were artificially inseminated by each bull with cryopreserved semen. Cows were selected، followed by checking the uterus and ovaries before insemination. Pregnancy was confirmed by rectal palpation at 60–65 and 85–90 days post-insemination. The semen straws that cryopreserved for three years were divided into three groups according to the bull's fertility: medium fertility (< 65%، MF، n=6)، the second of good fertility (65-79%، GF، n=7)، and the third of high fertility (>80%، HF، N=9).

Semen characteristics

Semen straws were thawed in a water bath (37ºC for 30 seconds). Semen characteristics were evaluated after thawing (sperm's cell individual motility، live sperm، abnormal sperm، sperm's plasma membrane and acrosome integrity، DNA damage). The seminal plasma was separated from straw semen by centrifugation(3000 rpm for 20 min.) for total antioxidant capacity، malondialdehyde concentration، and amino، fatty، and carboxylic acid determinations.

Determination of amino، fatty، and carboxylic acids concentrations: Some amino acids concentration (alanine، glycine، leucine، serine، proline، asparagine، aspartic، methionine، glutamic، tryptophan، and lysine) in the seminal plasma were estimated using high-performance liquid chromatography (1).===== The fat seminal plasma extraction was calculated using the AOAC method (13). Some fatty acid and carboxylic acid concentrations (acetic، butyric، propanoic، palmitic، oleic، linoleic، stearic، linolenic، arachidic، myristic، rvonic، cisdocosadienoic، tricosanoic، erucic، undecanoic) in seminal plasma were estimated using gas chromography (GC-2010، 17)..

Statistical analyses.

Statistical computations were carried out using the General Linear Model procedure in the SAS program، using CRD to examine the influence of sperm fertility on semen characteristics and some amino، fatty، and carboxylic acid concentrations in seminal plasma. The statistical model for the analysis of variance was as follows:

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

Where:

Y_{ij} = dependent variable (semen characteristics)

μ= Overall mean

G= Effect of sperm fertility (MF، GF، and HF groups).

RESULTS AND DISCUSSION

Semen characteristics

Non-significant differences were observed among the three groups in sperm cell individual motility، freezability، total sperm abnormalities، acrosome، plasma membrane integrity، total antioxidant capacity، DNA damage، and malondialdehyde concentration (Table 1). The HF group exhibited higher (P<0.01) fertility rates than MF and GF groups (Table 1).

Amino acids

There were no significant differences in most amino acid concentrations among the three groups (Table 2). The HF group recorded a higher glycine concentration (P< 0.05) than the MF group (Table 2).

Fatty and carboxylic acids

There were no significant differences in most fatty acid and all carboxylic acid concentrations among the three groups (Table 3). The HF (28.86±0.12%) and GF (28.73±0.09%) groups recorded a lesser (P<0.05) omega 9 concentration than the MF group (29.33±0.22%; Table3). The differences among the three groups in semen characteristics lacked significance (Table 1). These results are anticipated due to the higher sperm cell individual motility (>50%)، live sperm (>80%)، and lower abnormalities (<20%) of fresh and cryopreserved semen produced at the artificial insemination department. When assessed at 48 hours' post-cryopreservation، all semen samples will reject if the sperm cell individual motility is <40%، live sperm is <70%، and total abnormalities

are >20%. High-fertile bulls (HF) had the lowest decrease in their progressive motility at post-cryopreservation (higher freezability) as compared with fresh semen (13.99%). The values for individual progressive motility decreases for medium and good fertility are 17.45% and 16.66%، respectively. The semen cryopreservation processes are characterized by changes in osmotic pressure، ice granule formation، reactive oxygen species production، acrosome damage، DNA damage، and mitochondrial function damage. Mostek et al. (40) reported that fresh semen with high sperm progressive motility is more sensitive to cryopreservation due to adding carbonylated proteins (an irreversible oxidation process) in semen which is associated with energy metabolism and regulation of the sperm tail.

Table1.Effect some semen characteristics and biochemistry on Holstein bull sperm fertility (Mean ± SE).

Traits	Bulls Fertility			Level of sig.
	Medium fertility(<65%)	Good fertility (65-79%)	High fertility (>80%)	
Sperm individual motility (Fresh)	61.43±1.43a	60.00 ± 1.29a	58.13±0.91a	NS
Post-cryopreservation				
Sperm individual motility (%)	50.71± 0.71a	50.00 ± 1.29a	50.00 ± 0.94a	NS
Freezability	82.77±1.82a	83.42±2.02a	86.17±2.10a	NS
Live sperm (%)	75.43 ± 0.75ab	77.50 ± 0.67a	74.50± 0.82b	P≤0.05
Total sperm abnormalities (%)	18.43±0.99a	18.16±1.25a	17.13±0.91a	NS
Acrosome integrity (%)	86.14 ± 1.16a	85.50 ± 0.56a	85.25 ± 0.77a	NS
Plasma membrane integrity (%)	87.57± 0.84a	85.33 ± 0.71a	85.25 ±1.11a	NS
DNA damage (%)	7.82 ± 0.95a	8.53 ± 0.93a	8.39 ±0.66a	NS
Total antioxidants capacity (µg/dl)	41.65 ± 11.16a	46.86± 8.58a	75.93± 1.10a	NS
Fertility rate (%)	58.62±2.75c	74.72±1.11b	94.16±3.01a	P<0.01

Means with small superscripts within each row indicated significant differences among fertility group، NS: Non-significant، MDA: Malondialdehyde، sig.: significance.

The source of the carbonyl group is the oxidation of the unsaturated fatty acid process in sperm and seminal plasma. Carbonyl oxidation affects the fertility rate. The slightly increased freezability in the high-fertile bull group may be due to a significant increase in the concentration of glycine and the numerical of glutamic، alanine، and leucine (Table 2). Glutamine and glycerol work synergistically to protect sperm from the harmful effects of freezing. Argov-Argaman et al. (14) indicated that the deterioration of individual motility of bulls' sperm is due to a significant increase in the concentration of polyunsaturated and monounsaturated fatty acids in semen. The slightly increased freezability in the third group may be due to having a significantly lower concentration of omega-9 compared to the first group (Table 3). The efficiency of fertility assessment and semen cryopreservation depends on the better evaluation of the quality of both fresh and frozen semen، in addition، to detect of other components like proteins، minerals، fatty and amino acids (6،21،42). The fertility rate is affected by several factors، including nutrition، environmental conditions، heredity، hormones، stress، reproductive organ efficiency، health and body condition score، semen quality، and sexual receptivity (23،45،46). The fertility rate of Holstein bulls for the artificial insemination center was 75.17 percent (58.62-94.16%) in our current studies.

Table 2. Effect of Holstein bull fertility in some of semen amino acid concentration (Mean ± SE).

Amino acids (ppm)	Fertility			Level of sign.
	Medium (MF)	Good F(GF)	High F(HF)	
Total amino acid concentration	2388.17±87.86a	2475.75±166.7a	2648.3±144.17a	NS
Essential amino acid	1157.49±40.91a	1186 68±68.28a	1273.63±60.22a	NS
Non-essential amino acid	1230.69±47.92a	1289.07±98.71a	1374.76±84.55a	NS
Alanine	128.37± 8.01a	127.80± 18.90a	149.08± 12.3a	NS
Glycine	115.87± 7.58b	132.37±12.24ab	147.93± 10.4a	P<0.05
Valine	137.21± 6.55a	213.88± 17.81a	161.49± 10.39a	NS
Leucine	202.83±17.08a	152.73± 11.73a	216.26± 21.26a	NS
Serine	149.16± 7.165a	166.15± 10.77a	170.66± 10.32a	NS
Proline	190.22± 10.55a	189.53± 21.33a	202.69± 14.90a	NS
Asparagine	205.06± 8.44a	195.23 ± 14.60a	208.89 ± 18.54a	NS
Aspartic acid	245.09± 8.01a	276.03± 14.60a	275.29± 12.28a	NS
Methionine	194.80± 19.69a	194.80± 20.97a	219.3± 14.86a	NS
Glutamic acid	196.91± 10.27a	201.95± 16.15a	219.96± 12.52a	NS
Tryptophan	215.44± 5.63a	217.30± 11.90a	231.80± 9.26a	NS
Phenylalanine	209.9± 6.66a	212.02± 13.53a	233.8± 9.65a	NS
Lysine	197.27± 4.15a	197.37± 8.21a	210.93± 9.72a	NS

Means with small superscripts within each row indicated significant differences among fertility group، NS: Non-significant، sig.: significance.

Our fertility rate results for bulls were comparable to the previous study. Eidan (18) exhibited a 75-80% fertility rate for artificially inseminating cows with Holstein bull straw. Accurate selection of semen quality for cryopreservation in the artificial insemination center with an increased concentration of sperm in straw (15 million sperm per straw) led to an increase in pregnancy or fertility rates for cows inseminated by these straws. The differences in fertility among the three bull groups could attribute to the rich metabolites (amino، fatty، and carboxylic acids) and antioxidants in the semen of highly fertile bulls. These metabolites provide protection and nutrients to sperms during cryopreservation، thawing، transport، and fertilization. This indicates that there are other factors than the classical characteristics that are used in evaluating semen characteristics related to sperm fertility. Genetic or metabolite factors in semen may play a role in the fertility of bulls when semen is good at different

Table 3. Effect of Holstein bull fertility in some of semen fatty and carboxylic acid concentration (Mean ± SE).

Fatty acids (%)	Fertility(F)			Level of sign.
	Medium F.	Good F.	High F.	
Total fatty acid	70.19±1.78a	70.42±0.90a	70.36±1.09a	NS
Saturated fatty acid	31.32±0.79a	31.32±0.43a	31.56±0.53a	NS
Unsaturated fatty acid	38.87±1.0a	38.84±0.49a	38.80±0.57a	NS
Stearic	5.34± 0.23a	5.44± 0.12a	5.37± 0.12a	NS
Butyric	4.26± 0.10a	4.22± 0.04a	4.34± 0.09a	NS
Palmatic	7.23±0.10	7.42±0.0.08	7.39±0.11	NS
Myristic	8.36± 0.09a	8.33± 0.06a	8.34± 0.07a	NS
Arachidic	2.31±0.07a	2.43 ± 0.05a	2.41 ± 0.06a	NS
Tricosanoic	2.60 ± 0.09a	2.66± 0.07a	2.63± 0.08a	NS
Undecanoic	1.21± 0.09a	1.11± 0.02a	0.08± 0.03a	NS
Oleic	2.40± 0.06a	2.39± 0.03a	2.38± 0.03a	NS
Nervonic	245.09±8.01a	276.03± 14.6a	275.29±12.28a	NS
Erucic	5.21± 0.14a	5.23± 0.09a	5.16± 0.10a	NS
Linoleic	15.91± 0.41a	16.17 ± 0.17a	16.13± 0.15a	NS
Linolenic	0.67± 0.03a	0.67± 0.04a	0.63± 0.02a	NS
Cisdocosadienoic	1.71± 0.06a	1.76± 0.07a	1.73± 0.04a	NS
Omega 3	3.38 ±0.05a	3.45 ± 0.08a	3.36± 0.05a	NS
Omega 6	22.67± 0.15a	22.97± 0.10a	22.93± 0.10a	NS
Omega 9	29.33±0.22a	28.73±0.09b	28.86±0.12b	P<0.05
Total carboxylic acid	19.06± 0.32a	20.56± 1.46a	19.10± 0.17a	NS
Propionic	2.68± 0.12a	2.82±0.08a	2.75±0.06a	NS
Butyric	4.22±0.07a	5.61±1.40a	4.21± 0.06a	NS
Acetic	12.16± 0.13a	12.13± 0.13a	12.13± 0.10a	NS

Means with small superscripts within each row indicated significant differences among fertility group، NS: Non-significant، sig.: significance.

fertility bulls. (11، 23، 30). The current results revealed higher total antioxidants (Table 1) and total amino acid content with a significant glycine concentration increase (P< 0.05) in highly fertile bulls than in medium and good-fertile bulls (Table 2). Antioxidants play a role in scavenging ROS and controlling the lipid oxidation processes of the sperm and seminal plasma. Antioxidants maintain the ability of sperm to fertilize. There were small quantities of antioxidants in fresh semen (23). As a result، many studies have suggested the importance of adding antioxidants to sperm extenders. (2، 3، 4، 5، 13، 19، 22، 29، 38، 39). Seminal plasma amino acids have various functions، including reducing free radicals، protecting cells from protein denaturation، and providing an oxidative substrate for sperm. The results showed no significant differences for most acid concentrations in the seminal plasma of the bull groups. Tryptophan، leucine، phenylalanine، methionine، lysine، glutamic acid، aspartic acid، and serine had higher numerical concentrations. Glycine had a higher (P<0.05) concentration in HF bull's seminal plasma (Table 2). The highest fertility was in the third group، perhaps due to having more amino acids، followed by good and then medium bull fertility. Ugur et al. (47) showed that glutamic acid was the most abundant amino acid in the seminal plasma of bulls (3366± 547.3 nmol) compared to the rest of the amino acids estimated. The current results were consistent with those of Abdulkareem et al (6)، who found numerical increases of valine، serine، glutamic acid، and glycine in bulls with high sperm freezability (91.20%) compared to those of good (83.76%) and medium freezability (73.22%). Glutamic acid plays a crucial role in sperm metabolism during the production of ATP as a source of energy for sperm. Glutamic acid is necessary to protect the sperm membrane from lipid peroxidation damage and scavenges ROS from the sperm membrane. Glutamic acid، glycine، and cysteine are the main constituents of glutathione، which is a major antioxidant that maintains the redox balance within cells and inhibits cell damage resulting from lipid peroxidation and ROS production (47). Serine has been stimulating glutathione synthesis (31)، and indirectly through its role in glycine and cysteine formation (49)، serine also plays a role in stimulating genes and proteins that protect cells from oxidative stress. Glutamic acid، serine، and glycine play a critical role in the antioxidant production of bull semen. The current study recorded a higher numerical antioxidant concentration in HF bulls than in MF and GF bulls. Some amino acids have a role in energy production (gluconeogenesis)، such as methionine، cysteine، taurine، phenylalanine، valine، alanine، asparagine، glutamine، chlorine، proline، and aspartic acid (37). Further، proline، glycine، and carnitine have a significant biological role in preventing cell damage during the stress of cryopreservation (2، 50). The presence of proline in the seminal plasma improves sperm motility and fertilization (50). Proline increased by 6.6 and 6.9% in the HF group compared to the GF and MF groups (Table 2). Amino acids proteins play a key role in the function and characteristics of the sperm membrane and facilitate sperm-egg penetration. As a result، amino acids، particularly those found in seminal plasma، are good predictors of bull fertility (25). The AI with semen straws from HF bulls، characterized by an abundance of essential and non-essential amino acids in the seminal plasma، may increase the number of amino acids in the uterine cavity. The amino acid has an importance in the growth and development of embryos. Embryos depend on the nourishment of uterine secretions. Amino acids are the crucial components of uterine secretions. They contribute to protein synthesis and the regulation of the growth، development، and implantation of embryos in the uterus (35). Many essential amino acids (arginine، histidine، isoleucine، lysine، methionine، phenylalanine، valine، and threonine) and non-essential amino acids (alanine، asparagine، glutamine، chlorine، proline، serine، tyrosine، taurine، and hydroxyproline) were detected in the uterine cavity after fertilization and in the different stages of the estrus cycle. Some amino acids like glycine، glutamine، asparagine، phenylalanine، valine، proline، serine، aspartic acid، arginine، histidine، threonine، and alanine can serve as an energy source (gluconeogenesis). Meier et al. (35) showed a

numerical increase in leucine، serine، glycine، glutamine، alanine، and valine concentrations in the uterine fluids of fertile cows compared to those of low fertility (sub-fertile). They also indicated that most of the essential and non-essential amino acids decreased in the uterine cavity of pregnant cows compared to non-pregnant cows and before the embryos' implantation in the uterus due to their intake by the fetus. It was worth that glycine and glutamine play a significant role in increasing the cleavage rate and the number of blastocysts (33.53 vs. 27.09%) in the in vitro fertilization of bovine embryos (51). On the other hand، glycine، glutamine، histidine، and proline are functional as protective factors against freezing damage. They inhibit hydrogen peroxide and neutralize the mechanical osmosis of sperm. Accordingly، adding glycine، glutamine، alanine، and cysteine to semen extenders before cryopreservation improves sperm cell individual motility. Glycine is synthesized from choline، serine، hydroxyproline، and threonine. It is considered an antioxidant and anti-inflammatory and protects cellular and immune tissues (10). Serine is a cofactor with the sulfur transporter enzyme in the glutathione synthesis process. Both glycine and serine are significant for their roles in the glycolysis process in mitochondria. The mechanism by which proline works is due to its secondary amino composition. The pyrrolidine present in proline، distinguished by its low ability to provide an electron، has the primary advantage of scavenging ROS in animal cells، including sperm. Adding proline to the semen increases proline dehydrogenase activity (PDA). The PDA is located at the acrosomal cap and the midpiece of the sperm tail (50). Proline could remove the harmful effect of hydrogen peroxide، which reduces the sperm's progressive motility. Proline protects spermatozoa from oxidative shock during cryopreservation (50). It acts as a secondary compound and precursor of the enzyme proline dehydrogenase. Proline dehydrogenase enzyme converts proline to glutamate in several stages (41) with cofactors Δ1-pyrroline-5-carboxylate dehydrogenase (P5CDH). Amino acids work to stabilize and maintain the structure and function of sperm during freezing. Mohammed et al. (39) pointed out that adding 40 mmol/L glutamic acid to Tris extender resulted in superiority ($P< 0.05$) in the individual motility and live sperm of Holstein bulls post-cryopreservation، as well as an increase in the percentage of the sperm acrosome integrity and a decrease total sperm abnormalities post-cryopreservation. The metabolism of alanine، aspartate، and glutamine is associated with different cellular processes and sperm vital activities (37). The highest estimated concentration of glutamic acid in seminal plasma was found in Holstein bulls with HF، then GF and MF. These results are consistent with what was found by Ugur et al. (47)، who showed that glutamic acid had the highest concentration in the seminal plasma of bulls (3366 ± 547.3 nmol). On the other hand، glutamic acid is necessary to protect the sperm cell membrane from damage by lipid peroxidation، as it is an effective antioxidant to remove free radicals from the sperm cell membrane. Glutamic acid is one of the main components of the glutathione compound، which works to inhibit cell damage resulting from lipid peroxidation and the production of reactive oxygen species. The glutamic acid concentration in seminal plasma is a vital indicator for a selection bull with a high fertility rate. The lack of significant differences in amino acid concentrations between the three groups of bulls is most likely because the other standard amino acids were unavailable for evaluation. Highly fertile bulls had the lowest ($P<0.05$) concentration of omega-9 compared to the medium fertility group (Table 3). Argov-Argaman et al. (14) pointed out that the high concentration of polyunsaturated and monounsaturated fatty acids in bull sperm significantly ($P<0.01$) reduces sperm motility. Our results somewhat agreed with Argov-Argaman et al. (14) discovered no significant differences in most fatty acid concentrations، except for arachidonic acid، which was significantly decreased ($P <0.01$) in seminal plasma of bulls with different sperm motility percentages. Moreover، the current results were slightly consistent with Eidan et al. (21)، who indicated non-significant differences in the concentration of linoleic، palmitic، arachidonic، oleic، citric، linolenic، and

linoleic fatty acids among medium، good، and high freezable bulls. Martínez-Soto et al. (32) indicated that low temperatures change the semen lipid and antioxidant components to make them more viable during freezing and thawing. Polyunsaturated fatty acids are related to the motility and viability of sperm post-cryopreservation and thawing processes. At post-cryopreservation and thawing، an adverse effect of omega9 was observed on sperm cell individual motility and viability. The lack of significant differences in fatty acid concentrations among the three groups of bulls is most likely because the other standard fatty acids were unavailable for evaluation. There were no significant differences in carboxylic acid concentrations between the three groups (Table 2). These carboxylic acids are volatile fatty acids that ruminants depend on as an energy source. The lack of significant differences in carboxylic acids among the three groups of bulls may indicate that the energy level in the diet was balanced. As a result، the bulls produced good-quality fresh semen and maintained their quality post-cryopreservation (Table 1، 2). The high-energy diets hurt the quality of bull sperm produced (16). A high-energy diet causes increased adipose tissue deposition in the scrotal neck، which weakens thermoregulation in the scrotal region. High testicular temperatures reduce sperm production efficiency، viability، and fertility. There were no significant differences in fresh semen characteristics or post-cryopreservation among the three groups of bulls. The energy consumed was balanced for semen production. The lack of significance in carboxylic acid concentrations may return to the other undiagnosed carboxylic acids. In conclusion، the glycine and omega-9 in the seminal plasma of bulls can be used as indicators of their fertility. Carboxylic acid is a good indicator of semen quality or diet energy level. Moreover، other metabolic indicators like amino، fatty، and carboxylic acids appeared in the chromatographic analysis but were undiagnosed.

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