

## SOME FATTY ACID AND SEMEN CHARACTERISTICS OF HOLSTEIN BULLS AS INLUENCED BY DIFFERENT SPERM FREEZABILITY

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### ABSTRACT

This study was conducted to investigate the relationship between the concentration of some fatty acids in seminal plasma and semen characteristics of Holstein bulls with different sperm freezabilities. The semen straws that cryopreserved for two years were divided into three groups according to the sperm's freezability: medium (MF,73.02%), good (GF,83.76%), and high (HF,91.20%). The sperm's cell individual motility (SCIM) in fresh semen was superior ( $P \leq 0.05$ ) in bulls with MF and GF as compared with HF bulls. Concomitantly, the HF bulls were significantly ( $P \leq 0.05$ ) superior to those MF bulls in total fatty acid concentrations. Moreover, HF bulls recorded lesser ( $P \leq 0.05$ ) DNA damage percentage as compared with those of GF bulls. The differences among the three groups in concentrations of some fatty acid and SCIM at 48 hours and two years post-cryopreservation lacked significance. In conclusion, greater SCIM in bull's fresh semen recorded the lowest freezability, which indicated more sensitivity to freezing conditions and may reflect fertilizing ability of these bulls.

**Keywords:** sperms quality, metabolic indicators, bulls.

عیدان وآخرون

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بعض الاحماض الدهنية وصفات السائل المنوي لدى ثيران الهولشتاين بتاثيرقابليات تجميد مختلفة للنطف

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باحث

استاذ

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أجريت هذه الدراسة بهدف بيان بين تركيز بعض الأحماض الدهنية في البلازما المنوية كدلائل أيضية و صفات السائل المنوي لدى ثيران الهولشتاين بقابليات تجميد نطف مختلفة. تم استعمال قصبات السائل المنوي المحفوظة بالتجميد لمدة سنتين التي قسمت الى ثلاث مجاميع حسب قابلية التجميد: متوسطة (73.02 %)، جيدة (83.76 % ) وعالية (91.20 %) تفوقت النسبة المئوية للحركة الفردية للنطف في السائل المنوي الطازج ( $P \leq 0.05$ ) لدى الثيران ذات قابلية التجميد المتوسطة والجيدة مقارنة مع الثيران ذات قابلية التجميد العالية ، في حين تفوقت الثيران ذات قابلية التجميد العالية معنوياً ( $P \leq 0.05$ ) على نظيراتها ذات قابلية التجميد متوسطة في النسبة المئوية لسلامة الغشاء البلازمي للنطف وتركيز الأحماض الدهنية الكلية. كما سجلت الثيران ذات قابلية التجميد العالية أقل ضرر للمادة الوراثية معنوياً ( $P \leq 0.05$ ) مقارنة مع مجموعة الثيران ذات قابلية التجميد الجيدة. لم تكن هنالك فروق معنوية بين المجاميع الثلاث من الثيران في تركيز بعض الاحماض الدهنية والنسبة المئوية لحركة النطف الفردية بعد 48 ساعة وسنتين من الحفظ يمكن الاستنتاج ان الثيران التي تمتلك حركه نطف فردية عالية في السائل المنوي الطازج سجلت أقل قابلية تجميد مما يشير الى كونها اكثر حساسية لظروف الحفظ بالتجميد وربما ينعكس هذا على قابلية الاخصاب لدى هذه الثيران.

الكلمات المفتاحية: نوعية النطف، دلائل ايضية ، ثيران.

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## INTRODUCTION

The cow pregnancy rate increases with the number of progressively motile sperm, an increase in straw for artificial insemination, and depending on the freezability of sperm (29). Sperm freezability per bull varies by semen components, diluent types, and additives (20, 21, 44). Definition of freezability: the ratio of individual sperm motility after cryopreservation to individual sperm motility in fresh sperm (21). Sperm freezability is low due to high sperm fluidity after cryopreservation and thawing, which negatively affects sperm function and structure, changing sperm membrane permeability, individual motility, and viability. Sperm DNA is destroyed and its fertilization ability is reduced as a result of cold shock, osmotic pressure changes, lipoprotein re-proliferation in sperm membranes, and continuous stimulation. The re-proliferation of lipoproteins in sperm membranes, as well as the ongoing release of reactive oxygen species (ROS) by deformed, immature, and dead sperm, lower antioxidant levels in semen, exposing sperm to oxidative stress (1,23,31,32,37). Seminal plasma and extenders contain energy sources (fructose, glucose, sucrose), sperm protectants from the harmful effects of cooling, and freezing (glycerol and egg yolk), pH, osmotic regulators (sodium citrate, citric acid, etc.), antibiotics, antioxidants, and other substances that support sperm cryopreservation (18, 24, 25, 26, 42, 46). Artificial insemination technology has been commercially available to cattle breeders for more than sixty years (47), the main benefit of which is its ability to rapidly improve the herd genetics by taking advantage of elite bulls by purchasing semen straws from these bulls, which leads to improving the herd cows genetic and phenotypic traits and thus increasing the economic return for the breeders (8, 19, 32). The success of IVF is based on the quality of fresh semen and its ability to dilute and preserve with less loss of sperm motility, viability, and qualities after freezing and thawing (1,15). There are conflicting results of sperm cryopreservation for bull sperm, so the main challenge facing global straw semen production is its cryopreservation damage results (2, 6, 7, 14, 33). Seminal plasma

contains many chemical components like amino acids, proteins, fatty acids, organic acids, cholesterol, triglyceride alkaline phosphatase, Ca, Na, K, antioxidants ...etc. that are related to the quality of semen (4, 5, 22, 38, 39, 40). Fatty acids are present in the seminal plasma and the sperm membrane (4,5). They are associated with the phospholipid layer of the sperm membranes. Fatty acids are a source of energy, plasma membrane integrity-maintaining, fluidity, and permeability, especially under conditions of cold shock or cryopreservation (45). The sperm can use the fatty acids that surround the sperm to maintain their structures, viability, and function (17). Martínez-Soto et al. (35) indicated the possibility of using seminal fatty acids to predict cryopreservation ability in men. Many studies have shown the improvement of the cryopreservation ability and viability of the Holstein bull's sperm by adding fatty acids or omega-3 to the extenders (20, 25, 26, 27, 28). Argov-Argaman et al. (10), AL-Gebour found and Eidan (4,5) a change in the many fatty acids concentrations in seminal plasma and sperm between the winter and summer seasons. The improving bull's semen characteristics in winter with the increase in the concentration of Arachidic, Lignoceric, and Archodinic (seminal plasma, sperm tail, and sperm) Oleic (sperm), and Linoleic, Palmitic, Stearic, and Linolenic (sperm tail) as compared to the summer season. Argov-Argaman et al. (11) showed that Holstein bull's sperm phospholipid percentage had a significant effect on sperm function and motility. They also found a relationship between sperm individual progressive motility and the ratio of omega-6: omega-3, and monounsaturated fatty acids (MUFA) concentration. Kogan et. al., (29) indicated that semen with a high sperm progressive motility is more sensitive to cryopreservation processes due to the addition of carbonyl to sperm proteins as a result of semen unsaturated fatty acids oxidation. Because of the lack of studies on fatty acids concentration in seminal plasma with different bull freezability. So, this study was conducted to estimate some of the semen characteristics, and fatty acids concentration in seminal plasma as metabolic

markers and their relationships with Holstein bulls semen freezability.

## MATERIALS AND METHODS

### Experimental design

The study was carried out at the Artificial Insemination Department pertaining to the Directorate of Animal Resource, Ministry of Agriculture. Twenty-one Holstein bulls of 2-7 years old and 600-1000 kg body weight were used currently. The semen straws (0.25 ml) that cryopreserved for two years were divided into three groups according to the sperm's freezability: medium (69- 77% average = 73.0%, FA1, n=7), good (81-85 average =83.7, F2,n=9), and high (90-92 average =91.20%; FA3). All bulls were healthy, disease-free, and under constant veterinary supervision. All bulls were allocated on a standardized diet, as a concentrate ration was provided daily at a rate of 4-6 kg/bull. The ration consisted of 33% wheat, 35% barley, bran, 20% soybean meal, 10% maize, 0.5% CaCl<sub>2</sub>, 0.5% salt, and 1% vitamins, and minerals. Roughage consisted of alfalfa hay with an amount ranging between 7-9 kg/bull/day in addition to the green forage at a rate of 50-60 kg/bull/day. Fresh water and salt blocks were available ad libitum to the bulls.

### Determination of fatty acids concentrations.

The fat extraction in the seminal plasma was estimated according to the method of AOAC (9). The concentration of fatty acid (Arachidonic, linolic,  $\alpha$ -linolinic, oleic, stearic, and palmitic) in the seminal plasma was estimated using gas chromatography (GC-2010).

### Semen evaluation

Semen was collected from bulls using artificial vagina at one ejaculated/ bull. Semen was evaluated in terms of sperm's cell individual motility (%), live sperm (%), abnormal sperms (%), sperm's plasma membrane and acrosome integrity (%), DNA damage (%), total antioxidant capacity, malondialdehyde concentration (32, 36).

### 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 freezability on some fatty acid concentrations in seminal plasma and semen characteristics. The Statistical computations were carried out using General

Linear Model procedure in the SAS program, using CRD to examine the influence of sperm freezability on some of fatty acid concentrations in seminal plasma and semen characteristics. The statistical model for the analysis of variance was as follows:

$$Y_{ij} = \mu + G_i + e_{ij} \quad \text{Where:}$$

$Y_{ij}$  = dependent variable (Some of fatty acid or semen characteristics)

$\mu$  = Overall mean.

$G$  = Effect of sperm freezability (FA1, FA2, and FA3 groups).

$e_{ij}$  = error term. Differences among means were computed using the Duncan multiple range test.

## RESULTS AND DISCUSSION

### Semen characteristics and biochemistry:

The FA3 group exhibited lesser ( $P < 0.05$ ) sperm individual motility (Fresh semen), and DNA damage (post cryopreservation semen) than other groups (Table1). Non-significant differences among the three groups were shown in sperm individual motility, live sperm, total sperm abnormalities, acrosome integrity, total antioxidant capacity, and malondialdehyde concentration post cryopreservation of semen. The FA3 group revealed a significant difference ( $P < 0.05$ ) in plasma membrane integrity as compared with FA2, and FA1 groups (Table1). The sperm uses progressive motility to move within the reproductive female system for egg fertilization. Artificial insemination is prevalent in dairy cow herds, so the relationship between the sperm's progressive motility and their ability to cryopreserve is an important characteristic (35). Freezing and thawing processes are characterized by changes in osmotic pressure, ice crystal formation, and reactive oxygen species (ROS) formation. Also, DNA damage, plasma membrane integrity, intact acrosome, and mitochondrial function by preservatives from the cryoprotectants (3, 16). A lack of significant differences in sperm individual motility post cryopreservation among the three groups. This is because the bulls in the FA3 group had more intact plasma membranes than bulls in the FA2 and FA1 groups (Table 1), so, the FA3 group had more preservation and maintenance of sperm individual motility than others. Many researchers have pointed out the

positive relationship between sperm plasma membrane integrity, mitochondrial membranes, individual motility, and sperm vitality in bulls (13, 43). Another reason is that bull sperm cryopreservation has been reported to induce carbonylation (an irreversible oxidative modification) of proteins associated with energy metabolism and flagellum organization (29,37). These processes might explain the higher sensitivity of spermatozoa with high individual motility to cryopreservation. The oxidative stress imposed during the freeze–thaw process can induce structural and functional modifications in the high polyunsaturated fatty acid content of the membrane, which in turn might reduce the spermatozoa's ability to move straight forward (29). The superiority of the FA3 group in the plasma membrane integrity is due to possessing these bulls with the lowest dead sperm percentage (Table 1). Mandal et al. (34) indicated that cryopreservation and thawing of

sperm cause loss of viability, which leads to phospholipids peroxidation by dead sperm and damage to the sperm membrane. The reduced DNA damage to the sperm of the FA3 group may be due to the benefit of the antioxidants in semen, which protect them from oxidative stress damage caused by freezing and thawing processing (23,32, 36). The FA3 group recorded the lowest mathematical total antioxidant capacity ( $116.40 \pm 7.88 \mu\text{g/dl}$ ) than FA1 ( $137.26 \pm 9.44 \mu\text{g/dl}$ ) and FA2 groups ( $137.09 \pm 12.81 \mu\text{g/dl}$ ). It's well-known, that the priority of the semen antioxidants is to protect the genetic material in the nucleus before any other sperm part. DNA damage works to induce transcription factors that modify the process of proteins, fats, and other substance synthesis cells, as well as, induce the production of the substance that leads to pro-inflammatory, anti-inflammatory, and cytokines (12).

**Table1.Effect of Holstein bull sperm freezability in some semen characteristics and biochemistry (Mean  $\pm$  SE).**

Traits	Freezability			Level of sig.
	FA1	FA2	FA3	
Sperm individual motility (Fresh)	67.85 $\pm$ 1.48a	61.66 $\pm$ 1.44b	57.00 $\pm$ 1.22c	P<0.05
Post cryopreservation				
Sperm individual motility (%)	49.28 $\pm$ 1.30	51.66 $\pm$ 1.44	52.00 $\pm$ 1.22	NS
Live sperm (%)	78.42 $\pm$ 0.80	80.94 $\pm$ 1.07	81.10 $\pm$ 1.17	NS
Total sperm abnormalities (%)	17.02 $\pm$ 1.22	17.43 $\pm$ 1.21	17.18 $\pm$ 1.88	NS
Acrosome integrity (%)	91.14 $\pm$ 0.40	91.27 $\pm$ 0.54	90.50 $\pm$ 0.85	NS
plasma membrane integrity (%)	80.71 $\pm$ 0.83b	83.00 $\pm$ 0.64b	85.60 $\pm$ 1.17a	P $\leq$ 0.05
DNA damage (%)	8.44 $\pm$ 0.99ab	11.47 $\pm$ 1.50a	6.57 $\pm$ 0.80b	P $\leq$ 0.05
Total antioxidants capacity ( $\mu\text{g/dl}$ )	137.26 $\pm$ 9.44	137.09 $\pm$ 12.87	116.40 $\pm$ 7.88	NS
MDA concentration ( $\mu\text{m}/10^9\text{sperm}$ )	15.97 $\pm$ 2.29	16.01 $\pm$ 1.62	19.75 $\pm$ 2.59	NS

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

### Fatty acid concentrations

The FA1 group showed the greatest ( $P<0.01$ ) percentage in total concentration of fatty acids than the FA2 and FA3 groups (Table 2). Non-significant differences were detected in arachidonic, linoleic,  $\alpha$ -linolenic, oleic, stearic, and palmitic acid percentages among the three groups (Table 2). These results were in agreement with Argov-Argaman et al. (10), who found non-significance in arachidonic, linoleic, stearic, and palmitic acids in seminal plasma between the winter and summer seasons, despite significant differences between the two seasons in sperm individual motility. The sperm energy and some

structural molecules are provided by seminal plasma. The seminal plasmafat is directed to the various parts of the sperm according to the season and the nutrition available (30). The significant increases in sum fatty acids concentration in the FA1 may be due to the arithmetic increases in the concentration of all fatty acids in the current study. The linoleic and oleic fatty acids are important for the stability of the plasma membrane integrity of sperm (41). The high mathematical percentage of linoleic acid from the sum of fatty acids concentration in FA2 (42%) and FA3(41.7%) groups as compared with the FA1(40.4%) group may be related to the activation of the

sperm's individual motility post-cryopreservation and thawing (35). The desaturase activity of saturated fatty acids such as palmitic in seminal plasma, sperm, and accessory sex glands is evidence of the changes that occur during the summer season. The desaturase activity directly affects bulls'

semen quality like sperm individual motility (10), and plasma membrane integrity post-cryopreservation. In conclusion, bulls that have high sperm individual motility in fresh semen are more sensitive to freezing, as they did not maintain their superiority post-cryopreservation.

**Table2.Effect of Holstein bull sperm freezability on some fatty acids percentages (Mean ± SE).**

Fatty acids	Freezability			Level of significance
	FA1	FA2	FA3	
Sum of fatty acids concentration (%)	56.0±0.68a	53.27±0.38b	54.27±1.05b	P≤0.01
Arachidonic (%)	2.24±0.11	1.92±0.06	2.10±0.18	NS
Linoleic (%)	22.81±0.16	22.39±0.07	22.61±0.20	NS
α-Linolenic (%)	0.70±0.04	0.56±0.04	0.64±0.06	NS
Oleic (%)	12.81±0.14	12.44±0.06	12.63±0.20	NS
Stearic (%)	6.93±0.13	6.58±0.08	6.75±0.20	NS
Palmitic (%)	9.70±0.12	9.37±0.06	9.54±0.18	NS

Means with small superscripts within each row indicated significant differences among freezability group, NS: Non-significant.

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