

**COMPARISON OF SEMEN QULAITY FOR THREE LINES OF HOLSTEIN BULLS: 1. SOME IMMEDIATE AND MICROSCOPIC CHARACTERISTICS**A. J. A. Al-Saedi<sup>1</sup>T. A. Abdulkareem<sup>2</sup>

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**ABSTRACT**

This study was conducted to compare the semen immediate and microscopic characteristics of the three lines of Holstein bulls. Twenty-one Holstein bulls were divided into three groups belonging to the three lines of Holstein bulls; born in Iraq (Australian origin; n = 8), resulting from embryo transfer technology (New Zealand origin; n = 7), as well as the first generation (F1) resulted from two parents obtained from embryo transfer technology in Iraq (New Zealand origin; n = 6). The ET and L groups showed greater ( $P \leq 0.01$ ) ejaculate volume, live sperms percentage, and plasma membrane integrity percentage compared to the F1 group of bulls. Moreover, ET bulls exhibited higher ( $P \leq 0.01$ ) sperm concentration than L and F1 bulls. The ET and F1 bulls were superior ( $P \leq 0.01$ ) to L bulls in the percentages of sperm's cell individual motility and normal sperms. The percentage of DNA damage was significantly ( $P \leq 0.05$ ) decreased in the F1 group compared to the L group but did not differ from those of the ET group. In conclusion, the ET and F1 Holstein bulls were superior to L bulls in most immediate and microscopic semen characteristics and their adaptation to the Iraqi environment.

**Keywords:** Semen attributes, Embryo transfer, Holstein, bulls.

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مقارنة نوعية السائل المنوي لثلاث خطوط من ثيران الهولشتاين 1. بعض الصفات الآتية والمجهريّة

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المستخلص

اجريت هذه الدراسة لمقارنة الصفات الآتية والمجهريّة للسائل المنوي لثلاث خطوط من ثيران الهولشتاين. استعمل في هذه الدراسة 21 ثور قسمت الى ثلاث مجاميع من ثلاث خطوط لثيران الهولشتاين هي المولودة في العراق (استرالية المنشأ؛ n=8) والناجمة من تقانة نقل الأجنة (نيوزلندية المنشأ؛ n=7) والجيل الأول (F1) الناتج من ابوين تم الحصول عليهما من تقانة نقل الاجنة في العراق (نيوزلندية المنشأ؛ n=6). اظهرت المجموعتين ET وL تفوقا عالي المعنوية ( $P \leq 0.01$ ) في كل من حجم القذفة والنسبة المئوية للنطف الحية وسليمة الغشاء البلازمي، كما أظهرت ثيران الهولشتاين ضمن مجموعة ET أعلى ( $P \leq 0.01$ ) تركيز للنطف مقارنةً بثيران المجموعتين L وF1. تفوقت مجموعتي ثيران الهولشتاين ET وF1 معنويًا ( $P \leq 0.01$ ) على ثيران مجموعة L في النسبة المئوية لحركة النطف الفردية والطبيعية. كما انخفضت النسبة المئوية لضرر المادة الوراثية معنويًا ( $P \leq 0.05$ ) لدى ثيران المجموعة F1 مقارنةً بالمجموعة L ولكنها لم تختلف معنويًا مع المجموعة ET. يمكن الاستنتاج بان ثيران مجموعتي ET وF1 قد تميزت عن ثيران المجموعة L في معظم صفات السائل المنوي وتكيفها للبيئة العراقية.

الكلمات المفتاحية: صفات السائل المنوي، نقل الأجنة، الهولشتاين، الثيران.

## INTRODUCTION

Fertility in cows is a multi-parametric phenomenon that depends on the use of good quality and quantity of semen as well as on the exact timing and method used to inseminate the cows, in addition to the appropriate management of the herd (37). For high fertility post artificial insemination, the semen straw must contain an adequate number of viable and high-quality sperms capable of inducing fertilization and primary nucleus formation (38). The frozen-thawed sperms must be normal, having intact plasma membrane, acrosome and DNA, active mitochondria, and rapid progressively motile capable of reaching the site of fertilization within the female reproductive tract (1,2). On the other hand, embryo transfer is one of the techniques that have been recently adopted in Iraq. It began in 2010 in cooperation with the Food and Agriculture Organization (FAO) by transferring the embryos of pure Holstein cows imported from New Zealand to obtain bulls that can be used efficiently for the artificial insemination of the cows in Iraq through collecting approved frozen straws. Iraq lacks pure and genetically superior bulls. The process of buying them from European and American countries was too expensive (\$20-25 thousand) and not adapted to the harsh Iraqi environment (3). The importing of frozen embryos with reasonable prices (\$500/fetus) reduced the costs of producing semen and getting bulls adapted to the Iraqi environment when they were born locally (3). Eighteen Holstein calves were obtained from embryo transfer, and 13 F1-calves from two parents resulted from embryo transfer. Some of these bulls were used in semen collection at the Artificial Insemination Department when they reached the age of sexual maturity (3, personal communication, 2021). Previous study dealings with the evaluation of semen characteristics for Holstein bulls born in Iraq compared with those of embryo transfer and F1 bulls was not carried out. Therefore, this study was designed to compare the semen immediate and microscopic characteristics of those three lines of Holstein bulls.

## MATERIALS AND METHODS

**Experimental animals and design:** The study was carried out at the Artificial Insemination

Department (AID) pertaining to the Directorate of Animal Resource, Ministry of Agriculture in the Abu Ghraib region (25 km west of Baghdad) during the period from 9/1/2020 to 1/7/2021. Twenty-one Holstein bulls of 2-7.5 years old and 650-1000 kg body weight were used currently. All bulls were healthy, disease-free, and under constant veterinary supervision of both the Artificial Insemination Department and the Directorate of Veterinary/Ministry of Agriculture. All bulls were allocated on a standardized diet, as a concentrate ration provided daily at a rate of 4-6 kg/bull. The ration consisted of 35% barley, 33% wheat bran, 10% maize, 20% soybean meal, 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/animal/day in addition to the green forage at a rate of 50-60 kg/animal/day. Salt blocks and freshwater were available *ad libitum* to the animals. The bulls were divided into three groups belonging to the three lines of Holstein bulls. The first line was those born in Iraq (Australian origin; n = 8). The second and third lines resulted from embryo transfer (New Zealand origin; n = 7), and F1 bulls from two parents originated previously from the embryo transfer technique in Iraq (New Zealand origin; n = 6).

### Semen collection and evaluation

Semen samples were collected from each bull for 15 weeks. Semen was evaluated in terms of ejaculate volume, sperms concentration, mass activity (26), sperm's cell individual motility (12, 38) and live sperm (35). Sperm's plasma membrane (20) and acrosome integrity (18), as well as DNA damage percentage (36) were also determined.

### Statistical analyses

Statistical computations were carried out using General Linear Model (GLM) procedure in the SAS program (27), using CRD to examine the influence of Holstein bull lines on semen immediate and microscopic characteristics. The statistical model for analysis of variance was as follows:

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

Where:

$Y_{ij}$  = dependent variable (semen immediate and microscopic characteristics)

$\mu$  = Overall mean

G= Effect of Holstein bull lines (L, ET and F1).

$e_{ij}$ = error term

Differences among means were computed using Duncan multiple range test (13).

## RESULTS AND DISCUSSION

### Ejaculate volume and sperm concentration

The L ( $6.09 \pm 0.25$  ml) and ET ( $6.54 \pm 0.55$  ml) groups exhibited higher ( $P \leq 0.01$ ) ejaculate volume than the F1 group ( $5.00 \pm 0.29$  ml), while non-significant differences were observed between L and ET groups (Table 1). The superiority of the L and ET groups may be attributed to the increased age of the bulls in the L groups ( $7.42 \pm 0.27$  years) and ET ( $5.06 \pm 0.85$  years) compared to the F1 group ( $2.37 \pm 0.21$  years). The ET group ( $1613.3 \pm 92.30$ ) recorded a highly significant ( $P \leq 0.01$ ) superiority ( $P \leq 0.01$ ) on both the L and F1 groups for sperm concentration ( $\times 10^6$  / ml), while the L group was significantly ( $P \leq 0.01$ ;  $1336.9 \pm 92.19$ ) superior to the F1 group ( $908.5 \pm 82.40$ ) for a similar trait (Table 1). To the best of our knowledge, this is the first study dealing with the comparing of semen quality for three lines of Holstein bulls either in Iraq or in the world. Fuerst-Waltl et al (16), and Murphy et al (24) found that older bulls produce more semen than their young ones. These might be due to physiological changes related to an increase in body mass (8) and the size and maturity of the testes and accessory sex glands as the age progress and the amount of semen produced increases consequently (29). Moreover, Snoj et al (31) and Suyadi et al (34) found a significant effect of age on the ejaculate volume of European bulls (*Bos taurus*) and Bali elite bulls in Indonesia,

respectively, by increasing ejaculate volume with their age up to 8-10 years and decreasing after that. On the other hand, increasing ejaculate volume in the L and ET bull groups may be due to the increase in sperm concentration of these two groups compared to the F1 group. The increasing maturity of the seminiferous tubules and interstitium of the testicular parenchyma and spermatogenesis with the advancing age of L and ET bulls (29) could increase the sperm output ejaculate volume of these bulls (Suyadi et al., 34). Moreover, the method of semen collection and sexual preparation of bulls before the collection process may have an effect in causing differences in ejaculate volume and sperm concentration among the three groups. This notion was confirmed by Schenk (28), who found that good sexual stimulation of the bull and false mounting have a significant role in increasing the ejaculate volume and sperm per ejaculate. Because L and ET bulls have a experience in semen collection and sexual stimulation than F1 bulls, we believe that due to this experience, they were able to produce larger ejaculate volume and a greater sperm concentration than their counterparts of F1 bulls. The results of the ejaculate volume for L and ET groups are in line with those obtained by Eidan et al (14) for similar Holstein bulls at the AID ( $6.00 \pm 0.41$  ml), while it converged with those reported by Sultan and Eidan (33) for similar bulls ( $5.16 - 6.48$  ml). On the other hand, the current study recorded a higher sperm concentration ( $908.5 - 1613.3 \times 10^6$ ) than those obtained by Eidan et al (2017), namely  $888.15 \pm 46.41 \times 10^6$

**Table 1. Ejaculate volume, sperm concentration, mass activity and sperm's cell individual motility percentages for three Holstein bull lines in Iraq (Mean  $\pm$  SE).**

Holstein bull lines	L	ET	F1	Level of significance
Ejaculate volume (ml)	$6.09 \pm 0.25$ A	$6.54 \pm 0.55$ A	$5.00 \pm 0.29$ B	$P \leq 0.01$
Sperm concentration ( $\times 10^6$ /ml)	$1336.9 \pm 92.19$ B	$1613.3 \pm 92.30$ A	$908.5 \pm 82.40$ C	$P \leq 0.01$
Mass activity (%)	$35.69 \pm 2.37$ A	$25.31 \pm 2.40$ B	$14.06 \pm 1.50$ C	$P \leq 0.01$
Sperm's cell individual motility (%)	$48.37 \pm 2.61$ A	$37.18 \pm 2.96$ B	$22.09 \pm 1.83$ C	$P \leq 0.01$

Means with different superscripts within each row differ significantly ( $P \leq 0.01$ ). L: Holstein bulls born in Iraq, ET: Holstein bulls from embryo transfer technology in Iraq, F1: Holstein bulls, the first generation of embryo transfer technology.

**Mass activity and sperm's cell individual motility:** The L group exhibited a higher ( $P \leq 0.01$ ) mass activity percentage ( $35.69 \pm 2.37\%$ ) as compared with the F1 and ET groups (Table 1). Moreover, the ET group recorded a higher ( $P \leq 0.01$ ) mass activity ( $25.31 \pm 2.40\%$ ) compared to the F1 group ( $14.06 \pm 1.50\%$ ; Table 1). On the other hand, the highest ( $P \leq 0.01$ ) sperm's cell individual motility was noticed for the L group ( $48.37 \pm 2.61\%$ ) compared to the F1 and ET groups (Table 1). Furthermore, the ET group recorded a higher ( $P \leq 0.01$ ) sperm's cell individual motility ( $37.18 \pm 2.96\%$ ) compared to the F1 group ( $22.09 \pm 1.83\%$ ; Table 1). The significant increase in the mass activity and sperm's cell individual motility in the L group compared to the ET and F1 groups may be due to the numerical decrease in the potassium concentration in their seminal plasma ( $122.87 \pm 8.99$  mg/dl) compared to the two ET groups ( $-12.6\%$ ;  $138.40 \pm 12.57$  mg/dl) and F1 ( $-25.7\%$ ;  $154.54 \pm 13.02$  mg/dl; unpublished data). This notion was confirmed by Bearden et al (10), who explained that potassium is a natural metabolic inhibitor for sperms. This inhibition may decrease the mass activity and sperm's cell individual motility percentages of sperms. On the other hand, the reason behind higher sperm's cell individual motility in the L group may be due to the lesser DNA damage percentage of this group ( $8.24 \pm 1.52\%$ ; Figure 1). It is worthy that sperm's DNA damage is one of the utmost factors that lead to a decrease in the sperm's cell individual motility percentage and thus, causes infertility for most mammals (15, 40). In this regard, Aydos et al (6) found a significant negative correlation coefficient ( $r = -0.248$ ) between sperm's forward motility and DNA damage percentage in men. Interestingly, it was observed that the DNA damage gives a higher specificity (93.3%) in predicting the fertilization rate compared to the sperm's progressive motility (77%) in men (30). The decrease in the percentages of mass activity (14-35%) and sperm's cell individual motility

(22-48%) of bulls may return to many reasons like deterioration of the nutritional status and management requirements during the past two years accompanied by the Covid-19 pandemic and complete closure of most life facilities and government institutions including AID. Decreasing the working hours of the workers to 50 % of full time led to the lack of proper management requirements for bulls during this period. Moreover, increasing in heat stress and climatic changes that occurred in Iraq during the past years, and the rise in temperatures to more than 40 degrees Celsius during the summer, had a significant impact on the deterioration of semen quality, mass activity, and sperm's cell individual motility percentage in particular. The current mass activity percentage for the L group ( $35.69 \pm 2.37\%$ ; Table 1) was higher than that obtained by Sultan and Eidan (33) for the similar bulls in the AID (31.66 - 33.12%). Concomitantly, the ET and F1 groups got lesser mass activity percentage (14.06-25.31%; Table 1) than what was obtained by similar authors. On the other hand, the L group have got higher sperm's cell individual motility percentage ( $48.37 \pm 2.61\%$ ; Table 1) than those obtained by Sultan and Eidan (33) for similar Holstein bulls (43.80 - 46.00%), while the ET and F1 groups got lesser percentages (22.09 - 37.18%; Table 1). The differences in management practices and feeding levels are the main reasons behind variance among studies.

#### **Live and normal sperms percentage**

The L group exhibited greater ( $P \leq 0.01$ ) live sperm percentage ( $79.36 \pm 1.79\%$ ) compared to the ET ( $72.93 \pm 2.26\%$ ) and F1 ( $63.39 \pm 1.46\%$ ) groups. Concomitantly, the ET group was superior ( $P \leq 0.01$ ) in this trait than the F1 group (Table 2). On the other hand, the ET and F1 groups recorded higher ( $P \leq 0.01$ ) normal sperm percentages ( $92.40 \pm 0.36$  and  $91.81 \pm 0.24\%$  respectively) compared to the L group ( $89.35 \pm 0.72\%$ ), with non-significant differences occurred between the two first groups (Table 2).

**Table 2. Live, normal, plasma membrane integrity and acrosome integrity percentages of sperms for three Holstein bull lines in Iraq (Mean  $\pm$  SE).**

Holstein bull lines	L	ET	F1	Level of significance
<b>Trait</b>				
Live sperm percentage (%)	79.36 $\pm$ 1.79 A	72.93 $\pm$ 2.26 B	63.39 $\pm$ 1.46 C	P $\leq$ 0.01
Normal sperm percentage (%)	89.35 $\pm$ 0.72 B	92.40 $\pm$ 0.36 A	91.81 $\pm$ 0.24 A	P $\leq$ 0.01
Sperm plasma membrane integrity percentage (%)	84.08 $\pm$ 1.23 A	82.06 $\pm$ 1.27 A	76.97 $\pm$ 1.12 B	P $\leq$ 0.01
Sperm acrosome integrity percentage (%)	85.03 $\pm$ 1.34	87.13 $\pm$ 0.71	83.93 $\pm$ 0.83	NS

Means with different superscripts within each row differ significantly (P $\leq$ 0.01). L: Holstein bulls born in Iraq, ET: Holstein bulls from embryo transfer technology in Iraq, F1: Holstein bulls, the first generation of embryo transfer technology. NS: Non-significant.

The normal sperm percentages for the three experimental groups are good (89.35 - 92.40%) in assessing the fertility of the bulls, despite the superiority of the ET and F1 group compared to the L group (Table 2). Herein, it was found that bulls characterized by 70% upward normal sperm and no more than 20% sperm abnormalities are considered fertile bulls that can be used efficiently in artificial insemination of cows (9). The normal sperm percentage reflects the status of the seminiferous tubules in the testicular parenchyma as well as the function of the epididymis responsible for the maturation and storage of sperm (22).

**Sperm's plasma membrane and acrosome integrity:** The ET and L groups recorded higher (P $\leq$ 0.01) sperm's plasma membrane integrity percentage (84.08  $\pm$  1.23 and 82.06  $\pm$  1.27% respectively) in comparison with the F1 group (76.97  $\pm$  1.12 %), however ET and L groups did not differ significantly (Table 2). On the other hand, acrosome integrity percentage did not significantly differ among bull lines, however, it tended to be higher in ET groups as compared with the L (+2.5%) and F1(+3.8%) groups (Table 1). The increase in sperm plasma membrane integrity in L bulls may give an indirect assessment of fertility as compared with the ET and F1 bulls. Herein, Januskauskas et al (19) and Morrell et al (23) reported that enhancement of sperm progressive motility increases the relative fertility for AI bulls. On the other hand, Giritharan et al (17) found a positive correlation coefficient (r = 0.26) between sperm's plasma membrane integrity and acrosome integrity, 4-hours post-capacitation, and cleavage rate of embryos. It is worthy that

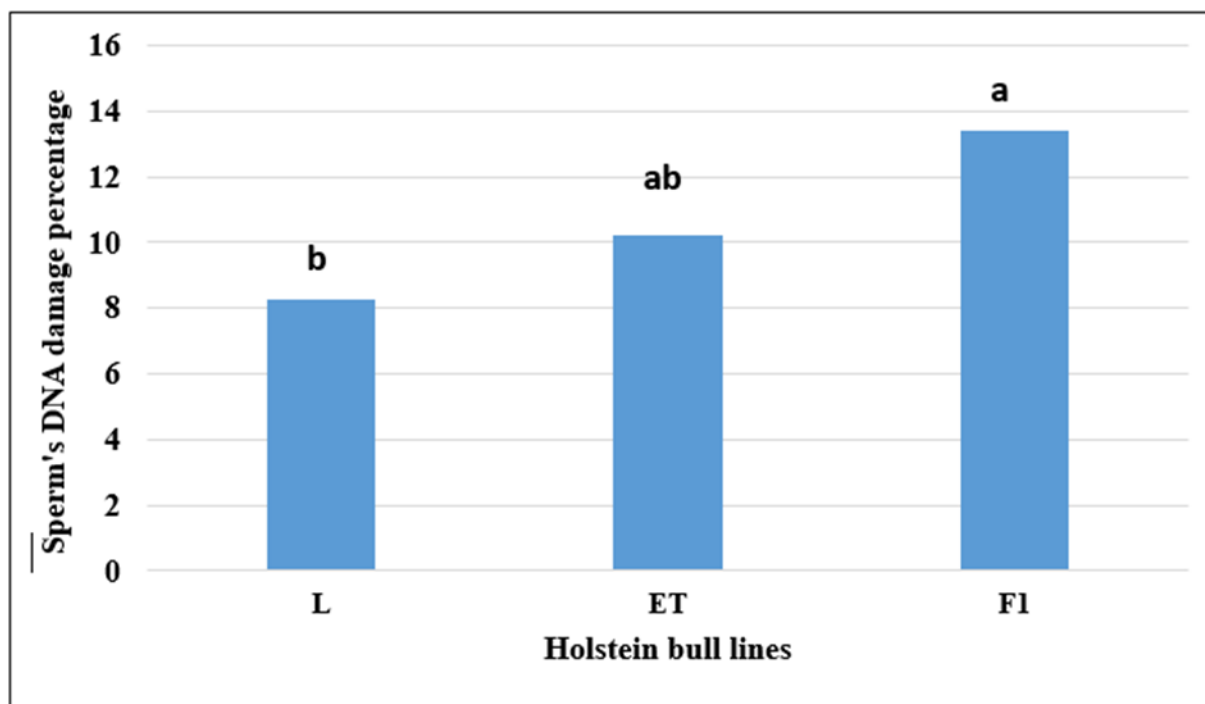
the current sperm's plasma membrane (76.97-84.08%) and acrosome integrity (83.93-87.13%) percentages were higher than those reported by Sultan and Eidan (33) for similar Holstein bulls at AID. They were 72.61-74.23% for plasma membrane integrity and 76.03-76.25% for acrosome integrity. These notions confirmed the higher fertilizing ability for AI bulls belonging to the ET and L groups. Also, the introduction of the new blood from these bulls through embryo transfer program have contributed effectively to increasing this fertility. Herein, the pregnancy rates of cows inseminated from these bulls reached 60-87% (4, 5, 32).

#### **Sperm's DNA damage percentage**

The L bulls exhibited a lesser (P $\leq$ 0.05) sperm's DNA damage percentage (8.24  $\pm$  1.52 %) compared to the F1 group, which recorded the highest value (13.37  $\pm$  0.99%). On the other hand, both L and ET groups did not differ with ET group (10.2  $\pm$  1.42 %) for the similar trait (Figure 1). The low percentage of sperm's DNA damage in the L bulls (8.24  $\pm$  1.52%; Figure 1) corresponds to the higher percentage of sperm's cell individual motility (48.37  $\pm$  2.61%; Table 1) and sperm's plasma membrane integrity percentage (84.08  $\pm$  1.23%; Table 2). Decreasing the DNA damage percentage for this group could be positively reflected in increasing the fertility rate of these bulls. Karoui et al (21) and Baiee et al (7) reported that bulls with high fertility have a lower DNA damage percentage than those with high values. These will increase the conception and pregnancy rates of inseminated cows from the semen of these bulls. One of the reasons behind the sperm's DNA damage is the generation of reactive oxygen species

(ROS) and its main components of free radicals, peroxides, and oxygen ions. These ROS generated during the spermatogenesis,

sperms maturation, and during freezing-thawing processes of the sperm (41).



**Figure 1. Sperm's DNA damage percentage for three Holstein bull lines in Iraq.**

Means with different superscripts differ significantly ( $P \leq 0.01$ ). It is noteworthy that calcium effectively contributes to protecting the DNA of the sperm, being the second messenger that regulates many cellular functions, including cell viability and cell death (11). Furthermore, calcium plays a pivotal role in the process of chromosome condensation necessary for the transferring genetic factors to the daughter cells during division, through its role in the continuation of the proper mitotic progression during the chromosome condensation after the breakdown of the nuclear envelope (25), and thus preservation of the sperm cell DNA.

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