EFFECT OF REDUCED GLUTATHIONE SUPPLEMENTATION ON MOTILITY, LIVABILITY AND ABNORMALITY OF HOLSTEIN SPERMATOZOA IN:
1. SEMEN STORED AT ROOM AND COOL TEMPERATURE
El-Sherbiny, M.A. *; M.H. El-Nenaey *; E.M.E. El-Siezy * and A.E. Abdel-Khalik **.

ABSTRACT

Semen was collected twice weekly from five sexually mature Holstein bulls raised at International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, to evaluate the effect of different concentrations of reduced glutathione (GSH) on sperm motility, livability and abnormality in semen stored at 25°C and 5°C for different storage periods. Only ejaculates with mass motility of 70% or more were pooled for each collection day for 5 weeks. The collected semen was diluted with Tris-extender and held in a water bath at 37°C, then divided into four portions. Different concentrations of GSH were added to the four diluted portions of semen (0, 0.4, 0.8 and 1.2 mM/ml Tris extender). Thereafter, each portion of semen was divided into two sub-portions, 10 replicates in each. Replicates of the 1st sub-portion were stored at 25°C (room temperature), while those of the 2nd portion were stored at 5°C (refrigerator temperature) for 0, 6, 24, 48, 72 and 96 h periods. Percentages of progressive motility, livability and abnormality of spermatozoa were determined at various storage times. Results revealed that adding GSH at concentration of 0.4 mM at 25°C showed the highest (P<0.05) sperm motility and livability percentages (56.8 and 57.1%, respectively), and the lowest (P<0.05) sperm abnormality (14.3%) as compared to the control without adding GSH (39.3, 40.8 and 17.3%, respectively). At different storage periods at 25°C, concentration of 0.4 mM GSH showed the highest motility and livability percentages, followed by 0.8 and 1.2 mM levels, respectively, while the control semen showed the lowest percentages. The opposite trend was observed for sperm abnormality. In semen stored at 25°C with 0.4 GSH, sperm cells maintained (P<0.05) their motility and livability only for 12 h; however, the differences in sperm abnormality were not significant between 0 and 48 h. In semen stored at 5°C, semen supplemented with 0.4 mM had the highest (P<0.05) sperm motility and livability and the lowest sperm abnormality. Semen supplemented with 1.2 mM, which was stored at 5°C showed the lowest sperm motility and livability. Sperm motility and livability decreased (P<0.05) by increasing storage period. However, sperm abnormality was not affected by storage period, ranging between 13.1-14.3%. By increasing storage period, sperm motility and livability gradually reduced. Semen supplemented with 0.4 mM showed almost the highest motility and livability percentages and the lowest sperm abnormality percentage. The present results suggested the possibility of storage bull semen at 25°C or 5°C for 48 h with maintenance of sperm motility and livability percentages above 50%, and sperm abnormality percentage at a level of 14% in semen supplemented with 0.4 mM glutathione.

Keywords: Bull sperm, glutathione, storage, motility, livability, abnormality.
INTRODUCTION

The cytoplasm of somatic cells contains several antioxidant enzyme systems, catalase, glutathione (GSH) and superoxide dismutase (SOD). However, sperm cells are devoid of most of this cytoplasm, so the antioxidant system in sperm cells of different species is weak (Li, 1975). Sperm cells have a high content of unsaturated fatty acids in their membrane. Therefore, they are susceptible to lipid peroxidation by H₂ and H₂O₂ (Alvarez and Storey, 1989 and Storey, 1997) and can readily undergo lipoperoxidation, particularly in the presence of oxygen (Foote et al., 2002).

One of the antioxidant substances that lacked in sperm cells is GSH, where one of its functions is to protect cells against the destructive effect of reactive oxygen species (ROS). Mammalian sperm vary, but tends to be low in GSH, glutathione peroxidase (GSP) and glutathione reductase (GPD), the key components in this protective system. The other antioxidants enzymes active in scavenging ROS are catalase and superoxide dismutase (SOD).

Under natural system of mating, sperm are exposed primarily to anaerobic conditions thus reducing potential damage by ROS. Also, fluids of oviduct contain substantial concentration of taurine (Miller and Shultz, 1987), an important protector of cells against accumulation of ROS when they are exposed to aerobic conditions (Alvarez and Storey, 1983a and Holmes et al., 1992).

Sperm cells used in AI are exposed to oxygen and visible light radiation during various processing procedure or in semen stored by cooling or at room temperature, which could lead to formation of ROS, and negatively affect sperm cell motility and genomic integrity (Aitken and Clarkson, 1988; Storey, 1997; Aitken et al., 1998 and Biodeau et al., 2001). Under these conditions, adding several types of antioxidants could help to maintain survival and motility of spermatozoa (Alvarez and Storey 1983a, b, 1989; Lindemann et al., 1988; Beconi et al., 1993; Lapointe et al., 1998; Biodeau et al., 2001 and Foote et al., 2002), when storage has been for several hours.

The objective of the current study was to testing the effect of adding different levels (0, 0.4, 0.8 and 1.2 mM) of reduced glutathione to Tris extender on motility, livability and abnormality of spermatozoa of Holstein bull semen stored at both room (25°C) and cool (5°C) temperatures for different storage times (0, 6, 12, 24, 48, 72 and 96 h).

MATERIALS AND METHODS

This study was carried out at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from 15 December 2005 to 30 January 2006.

Animals and management:

Five sexually mature Holstein bulls (3-5 years old and average LBW of 532±12.4 kg) raised at the ILMTC were used in this study. All bulls were free from any diseases with healthy appearance and were used for semen
collection as a routine work in ILMTC. Each bull was fed on daily ration composed of 8 kg concentrate feed mixture (CFM), 10 kg berseem (*Trifolium alexandrinum*) and 5 kg rice straw. Bulls were housed individually under semi-open sheds.

**Semen collection:**

Semen was collected twice weekly from each of the experimental bulls as a common practice in ILMTC using the conventional artificial vaginal method. One ejaculate from each bull (5 ejaculates for each run) was taken immediately to the laboratory and the ejaculates were pooled. Semen was collected before feeding at 8.00 a.m. A bull was used as teaser animal for sexual preparation.

Only ejaculates with mass motility of 70% or more were pooled for each collection day for 5 weeks. The collected semen was diluted with Tris-extender and held in a water bath at 37°C, then divided into four portions. Different concentrations of GSH were added to the four diluted portions of semen (0, 0.4, 0.8 and 1.2 mM/ml Tris extender). Thereafter, each portion of semen was divided into two sub-portions, 10 replicates in each. Replicates of the 1st sub-portion were stored at 25°C (room temperature), while those of the 2nd portion were stored at 5°C (refrigerator temperature) for 0, 6, 24, 48, 72 and 96 h periods.

Semen was diluted in at a rate of 1:20 in heated (37°C) Tris extender. The Tris extender was prepared with Tris (0.05 g), citric acid (3.35 g), streptomycin (0.5 g), lincomycin (0.01 g) and distilled water (100 ml) then 16 ml egg yolk, 12 ml glycerol and 72 ml distilled water were added to obtain 200 ml of the Tris extender, then it was mixed and kept at 37°C.

**Assessment and evaluation of semen:**

Semen of each GSH concentration was stored for 0, 6, 12, 24, 48, 72 and 96 h at room temperature (25°C) and refrigerator temperature (5°C). After each storage time at 25 and 5°C, percentage of progressive motility, livability and abnormality of spermatozoa in 10 replicates of each concentration were determined.

The percentage of motile spermatozoa (progressive motility) in each replicate was assessed using research microscope with warmed stage (37°C) under the high power magnification (x400) according to Amman and Hammerstedt (1980). Sperm livability percentage was determined using eosin and nigrosin mixture stain according to Hackett and Macpherson (1965). Live spermatozoa ( unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa. Then percentage of live spermatozoa was calculated. Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983).

**Statistical analysis:**

Results were statistically analyzed according to Snedecor and Cochran (1982) using SAS system (1985). The differences among means
were tested using new multiple range test (Duncan, 1955). The percentage values of sperm progressive motility, livability, and abnormality were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed

RESULTS AND DISCUSSION

Semen stored at room temperature (25°C):
Results presented in table (1) showed significant (P<0.05) effect of GSH supplementation on all sperm characteristics studied. Supplementation of 0.4 mM GSH showed the highest (P<0.05) sperm motility and livability percentages. However, all GSH supplements reduced (P<0.05) sperm abnormality as compared to the control (without additives), being the best in semen supplemented with 0.4 mM GSH.

Table (1): Effect of GSH supplementation and storage time on percentages of motility, livability and abnormality of Holstein spermatozoa in semen stored at 25°C.

<table>
<thead>
<tr>
<th>Effect of GSH supplementation (mM)</th>
<th>Motility (%)</th>
<th>Livability (%)</th>
<th>Abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39.3±3.06⁵</td>
<td>40.8±3.08⁵</td>
<td>17.3±0.28⁵</td>
</tr>
<tr>
<td>0.4</td>
<td>56.8±2.28⁴</td>
<td>57.1±2.21⁴</td>
<td>14.3±0.38⁵</td>
</tr>
<tr>
<td>0.8</td>
<td>48.1±2.76⁴</td>
<td>48.9±2.65⁴</td>
<td>15.1±0.41⁵</td>
</tr>
<tr>
<td>1.2</td>
<td>42.3±2.92⁴</td>
<td>43.0±2.80⁴</td>
<td>15.1±0.37⁵</td>
</tr>
</tbody>
</table>

Effect of storage time (h):

| 0                                  | 71.8±0.89⁴   | 71.2±1.34⁴     | 14.2±0.47⁴      |
| 6                                  | 68.1±1.16⁴   | 67.7±1.13⁴     | 14.6±0.61⁴      |
| 12                                 | 61.0±1.56⁴   | 61.7±1.49⁴     | 14.9±0.53⁴      |
| 24                                 | 50.1±2.04⁴   | 51.9±1.99⁴     | 15.1±0.53⁴      |
| 48                                 | 41.2±2.33⁴   | 42.0±2.38⁴     | 15.4±0.47⁴      |
| 72                                 | 23.5±2.35⁴   | 25.5±2.17⁴     | 16.6±0.38⁴      |
| 96                                 | 10.6±1.57⁴   | 12.6±1.67⁴     | 17.4±0.37⁴      |

*: Means denoted within the same column with different superscripts are significantly different at (P<0.05).

In semen supplemented with concentrations of 0.5, 1.0, 1.5 and 2.0 mM of GSH and stored at 25°C, Foote et al. (2002) found that addition of 1.0 and 1.5 mM of GSH in TALP-extender increased (P<0.05) sperm motility, being 52.0 and 51.0%, respectively as compared to 45.7% in the control semen (0 mM GSH). In the present study, the beneficial effect on sperm motility and livability percentages in semen supplemented with GSH was obtained for all GSH supplements, being the best for 0.4 mM of GSH (56.8 and 57.1%, respectively) as compared to the control semen (39.3 and 40.8%, respectively). The different trends of GSH supplements in our study may be due to type of the extender used in semen dilution. The later author found that the corresponding effect of GSH levels (0.5, 1.0, 1.5 and 2.0 mM) in whole milk (WVM) was not significant, but sperm motility was significantly
higher with WM than TALP extenders. Accordingly, it was suggested an
interaction effect between GSH level and type of extender. In this respect,
Raina et al. (2002) showed that sperm motility was affected significantly
(P<0.01) by extender–antioxidant combination.

Effect of storage period on all sperm characteristics studied was
significant (P<0.05). Sperm cells significantly (P<0.05) maintained their
motility and livability percentages only for 6 h, thereafter these percentages
significantly (P<0.05) reduced with increasing storage time, reaching the
minimum values at storage period of 96 h. On the other hand, percentage of
sperm abnormality did not differ significantly by increasing storage time from
0 up to 48 h, and then significantly (P<0.05) increased after 96 h (Table 1).

Raina et al. (2002) found that overall mean percent motility observed
after 0, 4, 8, 12 and 24 h of preservation at 37°C were 68.5, 58.9, 45.0, 38.1
and 18.1%, respectively. Type of antioxidant added and storage interval were
found to significantly (P<0.01) affect sperm motility on room temperature
preservation.

The effect of interaction between GSH level and storage period on
percentages of motility, livability and abnormality of spermatozoa was not
significant. Although, sperm motility and livability percentages in control and
all supplemented semen showed gradual reduction with increasing storage
time, semen supplemented with 0.4 mM GSH showed the highest values,
followed by those supplemented with 0.8 and 1.2 mM, respectively. While,
the control semen showed the lowest values (Figs. 1 and 2, respectively).
The opposite trend was observed for sperm abnormality percentages (Fig. 3).
Such trends indicated the possibility of maintaining percentage above
50% as motile and live spermatozoa with about 14% sperm abnormality in
semen diluted with Tris-extender supplemented with 0.4 mM GSH for 48 h.

Semen stored at cool temperature (5°C):

Results presented in Table 2 showed significant (P<0.05) effect of
GSH supplementation on all sperm characteristics studied. Supplementation
of 0.4 mM GSH at 0.4 mM significantly (P<0.05) showed the highest
percentages of motility and livability of sperm cells and the lowest sperm
abnormality percentage. Adding 1.2 mM of GSH to semen stored at 5°C
showed the lowest motility and livability percentages, being lower than those
in the control semen. This may suggest the toxic effect of this concentration
in semen stored at the cooler temperature than at 25°C.

During cooling to 4°C increasing production of ROS was measured
in human semen (Wang et al., 1997). Hydrogen peroxide (H₂O₂) as ROS has
been shown to decrease motility in mouse, human, bull, and rabbit
spermatozoa (Alvarez and Storey, 1989). Under these conditions, adding
several types of antioxidants could help to maintain survival and motility of
spermatozoa (Alvarez and Storey 1983a, b, 1989; Lindemann et al., 1988;
Beconi et al., 1993; Lapointe and Sirard, 1998; Bilodeau et al., 2001 and
Foote et al., 2002). These findings were indicated in our study when of 0.4
mM GSH was supplemented to semen, whereas percentages of sperm
motility and livability were 52.8 and 53.3%, respectively as compared to 41.6
and 43.0% in semen without supplementation.

1965
**Fig. (1):** Effect of GSH supplements on sperm motility percentage in semen stored for different times at 25°C.

**Fig. (2):** Effect of GSH levels on live sperm percentage in semen stored for different times at 25°C.

**Fig. (3):** Effect of GSH supplements on sperm abnormality percentage in semen stored for different times at 25°C.
The effect of addition of natural antioxidants into semen diluents on the preservability of buffalo semen was studied by Raina et al. (2002). At 4–7°C, overall mean of percent motility for semen extended in MYE, MYC, MYO, and TYE. TYC and TYO were 44.8, 42.7, 38.7, 36.0, 35.2 and 33.0%, respectively. The results showed that motility was significantly (P<0.01) affected by extender–antioxidant combination.

Also, the effect of storage time on all sperm characteristics studied was significant (P<0.05). Sperm motility and livability percentages significantly (P<0.05) decreased by increasing storage time. However, sperm abnormality percentage was not affected by storage period, ranging between 13.1-14.3% (Table 2).

Foote et al. (2002) found no effect of GSH addition (0.5 and 1.0 mM) in WM on sperm motility immediately after cooling to 5°C and then after 24 h storage at 25°C, a beneficial effect was detected for 0.5 of GSH level on sperm motility.

Table (2): Effect of GSH supplementation and storage time on percentages of sperm motility, livability and abnormality of Holstein spermatozoa in semen stored at 5°C.

<table>
<thead>
<tr>
<th>Item</th>
<th>Motility (%)</th>
<th>Livability (%)</th>
<th>Abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of GSH supplementation (mM):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>41.6±2.7</td>
<td>43.0±2.7</td>
<td>16.0±0.35</td>
</tr>
<tr>
<td>0.4</td>
<td>52.8±2.2</td>
<td>53.3±2.2</td>
<td>11.8±0.41</td>
</tr>
<tr>
<td>0.8</td>
<td>44.3±2.7</td>
<td>44.6±2.7</td>
<td>13.9±0.45</td>
</tr>
<tr>
<td>1.2</td>
<td>40.6±2.8</td>
<td>42.6±2.7</td>
<td>13.8±0.36</td>
</tr>
<tr>
<td>Effect of storage time (h):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>70.6±0.74</td>
<td>70.9±0.83</td>
<td>13.1±0.49</td>
</tr>
<tr>
<td>6</td>
<td>62.5±0.95</td>
<td>63.7±0.85</td>
<td>13.7±0.56</td>
</tr>
<tr>
<td>24</td>
<td>52.8±1.3</td>
<td>53.8±1.3</td>
<td>13.1±0.58</td>
</tr>
<tr>
<td>48</td>
<td>39.4±1.8</td>
<td>40.7±1.8</td>
<td>14.3±0.51</td>
</tr>
<tr>
<td>72</td>
<td>27.8±1.7</td>
<td>28.8±1.7</td>
<td>14.3±0.55</td>
</tr>
<tr>
<td>96</td>
<td>16.5±1.7</td>
<td>17.1±1.7</td>
<td>14.0±0.43</td>
</tr>
</tbody>
</table>

A, b and c: Means denoted within the same column with different superscripts are significantly different at (P<0.05).

The effect of interaction between GSH level and storage period on percentages of motility, livability and abnormality of spermatozoa was not significant. A gradual reduction in sperm motility and livability percentages was observed in control and semen supplemented with increasing storage time. At all storage times, semen supplemented with 0.4 mM of GSH showed the highest values, followed by those supplemented with 0.8 and 0 mM of GSH, respectively. While, semen supplemented with 1.2 mM showed the lowest values (Figs. 4 and 5, respectively). However, sperm abnormality percentages in supplemented and control semen showed gradual increase by increasing storage time, being the lowest in semen supplemented with 0.4 mM of GSH (Fig. 6).
Fig. (4): Effect of GSH supplements on sperm motility percentage in semen stored for different times at 5°C.

Fig. (5): Effect of GSH supplements on live sperm in semen stored for different times at 5°C.

Fig. (6): Effect of GSH supplements on sperm abnormality in semen stored for different times at 5°C.
Such trends indicated the possibility of cooling bull semen to 5 °C for 48 h with appropriate percentages of motility, livability (>50%) and abnormality (12%).

In conclusion, supplementation of semen diluted with Tris-extender with 0.4 mM of GSH resulted in bull spermatozoa to maintain their motility and livability above 50% and abnormality percentage less than 15% at room temperature (25°C) or at cooling temperature (5°C) for 48 hours.

REFERENCES


El-Sherbieny, M.A. et al.


تأثير إضافة الجلوتاتيون المخفض على حيوية وشواذ الحيوانات النموية

لطلق الهولستين: السائل المنوي المحسن على درجة حرارة الغرفة

(دارجة مؤوية) والمريس على 0 درجات مؤوية

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في هذه الدراسة تم جمع السائل المنوي مرتين أسبوعياً باستخدام آلية التخمير الشعاعية من خمسة طبقات خشبية، ناضجة جامياً (عبراً من 3 إلى 5 سنوات ونسبة سلالة نورت / كر ו.ك.) وذلك لتقييم احصاء النتائج المختلفة من الجلوتاتيون كOTORCT: كتابة النتائج الإجمالية على حيوية وشواذ الحيوانات المنوية، وتم تحليل النتائج النهائية للخصائص في مجموعة مكونة من خمسة أفراد، وتم استخدام عبارة عبارة熔منظمات المول组合别27. بعد ذلك، قسمت إلى اربع عبارة معناية تحاكي لكل عملة طريقة فصول (كانور). بينما ثلاث عبارة أخرى كانت عبارة تصلب الجلوتاتيون بتركيز (أ) 0.4 مل. مول /س (0.8 %) و (ب) 0.2 مل. مول /س و (ج) 0.1 مل. مول /س. ولكن معذب النتائج في النتائج 2004.

تم تضييق السائل المنوي لكل عملة لمدة صغر من مدة 30 دقيقة، وذلك على درجة حرارة 41.50 مل. مول لكل عملة المنوي المحسن على درجة حرارة 0.50 مل. مول إلى السائل المنوي المحسن على درجة حرارة 0.20 مل. مول على كل من الحيوانات المنوية ونسبة الحيوانات المنوية جيدة، حيث تبين جيدة حيوية وشواذ سنا. من ناحية أخرى فإن نسبة الحيوانات المنوية الشاذة تم تخفيف معنوي بين صغر من 0.40 مل. مول إلى 0.20 مل. مول، وتمت كل التصوير، وتم إعلان قيمة ترتيب، بينما اعلان قيمة في السائل المنوي المحسن.

1. حافظت الحيوانات المنوية على حيويتها وشواذها حتى من النذير بعد ذلك، انخفضت نسبة الحيوانات المنوية الحية وشواذ مع طول فترة التخزين ووصلت إلى أدنى قيمة عند 96 ساعة. ومن ناحية أخرى فإن نسبة الحيوانات المنوية الشاذة تم تخفيف معنوي بين صغر من 0.40 مل. مول إلى 0.20 مل. مول، وتمت كل التصوير، وتم إعلان قيمة ترتيب، بينما اعلان قيمة في السائل المنوي المحسن.

2. كانت القدرة على حيوية وشواذ الحيوانات المنوية أعلى قليل من نسبة الحيوانات المنوية сигма. في السائل المنوي المحسن على درجة حرارة 0.50 مل. مول، حيث تم تخفيف معنوي بين صغر من 0.20 مل. مول إلى 0.10 مل. مول، وتمت كل التصوير، وتم إعلان قيمة ترتيب، بينما اعلان قيمة في السائل المنوي المحسن.

3. أظهر السائل المنوي المحسن على درجة حرارة 0.50 مل. مول على الأقل، في الحيوانات المنوية المنوية، حيث تم تخفيف معنوي بين صغر من 0.20 مل. مول إلى 0.10 مل. مول، وتمت كل التصوير، وتم إعلان قيمة ترتيب، بينما اعلان قيمة في السائل المنوي المحسن.

4. أظهر السائل المنوي المعالج على صغر معنوي بين 0.20 مل. مول على الأقل، في الحيوانات المنوية، حيث تم تخفيف معنوي بين صغر من 0.10 مل. مول إلى 0.05 مل. مول، وتمت كل التصوير، وتم إعلان قيمة ترتيب، بينما اعلان قيمة في السائل المنوي المحسن.