

Malondialdehyde Level in Seminal Plasma of Cryopreserved Holstein Bull Semen after Addition of Zinc, Cysteine, Prostaglandin F2 α and their Combination *in vitro*

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Abstract

The study was conducted to know the level of Malondialdehyde (MDA) in seminal plasma of cryopreserved semen of Holstein bulls after addition of zinc sulphate, cysteine, PGF2 α and their combination *in vitro*. Semen was collected from 7 Holstein bulls, presented in Artificial insemination Center which belonged to the Directorate of Animal Resources/ Ministry of Agriculture at Abu-Graib at the west of Baghdad. Pooled semen were diluted with Tris- based extender and divided into five parts. The first part (T₁) serve as a control (without addition). The 2nd part (T₂) added to it zinc sulphate (0,576 mmol/ml). The 3rd part (T₃) added to it cysteine (5 mmol/ml). The 4th part (T₄) added to it PGF2 α (37.5 pg/ml). while the 5th part added to it a combination of previous substances at the same concentration. They packed in straws and cryopreserved in a liquid nitrogen and after 30, 60 and 90 days. Seminal plasma when isolated to measure the level of MDA. The results showed a significant decrease (P>0.01) in MDA level in the combination treated group (zinc, cysteine and PGF2 α) 0.450 \pm 0.11 (mmol/ml) as compared with control group 1.025 \pm 0.38 (mmol/ml), zinc 0.867 \pm 0.12 (mmol/ml), cysteine 1.06 \pm 0.12 (mmol/ml) and PGF2 α group 0.968 \pm 0.17 (mmol/ml) respectively. It was concluded from this study that addition of a combination of zinc, cysteine and PGF2 α to the Holstein bull semen could decrease the level of MDA which might be due to the synergistic effect of these substances.

Keywords: MDA, Zinc, Cysteine, PGF2 α , Holstein bulls, Semen, Cryopreservation.

مستوى المألون ثنائي الديهايد في بلازما السائل المنوي المجمد لثيران الهولشتاين بعد إضافة الزنك، السستين، هرمون وخليطهما مختبرياً F2 α البروستكلاندين

الخلاصة

أجريت الدراسة لمعرفة مستوى المألون ثنائي الديهايد في بلازما السائل المنوي المجمد لثيران الهولشتاين بعد إضافة الزنك، السستين، هرمون PGF2 α وخليطهما مختبرياً. تم جمع السائل المنوي من 7 ثيران من سلالة الهولشتاين تواجدت في مركز التلقيح الصناعي التابع لمديرية الثروة الحيوانية/ وزارة الزراعة- ابو غريب- بغداد. أخذ مزيج السائل المنوي (Pooled) وخفف بالترس وقسم إلى 5 أجزاء الجزء الأول (T₁) اعتبر معاملة سيطرة (بدون إضافة). الجزء الثاني (T₂) أضيف له سلفات الزنك (0.576 ملليمول/مل). أما الجزء الثالث (T₃) أضيف له السستين (5 ملليمول/مل). الجزء الرابع (T₄) أضيف له هرمون PGF2 α (37.5 بيكوغرام/مل). أما الجزء الخامس (T₅) فأضيف له خليط من المواد المذكورة أعلاه وبنفس التراكيز. تم وضع السائل المنوي في قصبات وجمد بالنتروجين السائل وبعد مدد 30، 60 و90 يوماً تم فحص المألون ثنائي الديهايد عن طريق فصل بلازما السائل المنوي. أظهرت النتائج انخفاض معنوي (P>0.01) في تركيز المألون ثنائي الديهايد في مجموعة المعاملة بخليط الزنك، السستين وPGF2 α 0.450 \pm 0.11 (ملليمول/مل) عند مقارنته مع مجاميع السيطرة 1.025 \pm 0.38 (ملليمول/مل)، والزنك 0.867 \pm 0.12 (ملليمول/مل) والسستين 1.06 \pm 0.12 (ملليمول/مل) وPGF2 α 0.968 \pm 0.17 (ملليمول/مل) على التوالي. وقد استنتج من الدراسة أن إضافة خليط من الزنك، السستين وPGF2 α للسائل المنوي للثيران يخفض مستوى المألون ثنائي الديهايد بسبب العمل التآزري لهذه الإضافات.

Introduction

In order to increase productive performance of animals for human food to face the increase in world population. We should increase the fertility of animals especially the cattle, which is one of solution of world problem. The know of mechanisms and challenges of reproductive biotechnologies was important to improve cattle industry. Artificial insemination (A.I.) was one of these technologies utilized in advance farm of cattle that assist in acceleration of genetic improvement and selection (1).

The successful cryopreservation of semen improve the efficiency of A.I. The methods used for cryopreservation of semen not always efficient due to that the large number of sperms suffer from functional damage that leads to the loss of fertility after cryopreservation and thawing (2, 3), During these processes sperms faces functional and structural challenges due to the changes in the osmotic pressure, oxidation stress and extracellular crystallization, So that the need of antioxidant and cryoprotactant agent addition to semen Malondialdehyde (MDA) constitute the final end product of lipid peroxidation. It formed as a results of conversion of un saturated fatty acid peroxide to MDA. It constitutes visceral the conditions of increases the formation of free radicals and oxidative stress (4, 5). Lipid peroxidation produces MDA that causes a damage of structure and function of plasma membrane of bull sperm cell (6, 7). The production of MDA in the semen have a direct relationship with the quantity of unsaturated fatty acids, types of reactive oxygen species in the semen (8). So that the level of MDA considered as an indicator to oxidative stress and infertility (7, 9). The study was aimed to show the level of MDA in bull semen after addition of Zn, cysteine and PGF2 α to the bull semen *in vitro* and cryopreserved and examined after 30, 60

and 90 days.

Materials and methods

The study was conducted on 7 Holstein bulls, aged 2.5- 3 years with a body weight 500- 750 kg/ bull, presented in the farm of Artificial insemination centers/ Directorate of Animal Resources/ Ministry of Agriculture at Abu- Graib, Baghdad. Semen was collected by Artificial Vagina one ejaculate per bull weekly during the period from Feb. 2019 to the end of Aug. 2019. Pooled semen were used semen were diluted with Tris- base extender, Then the diluted semen divided into five parts. The first part (T₁) serve as a control. The 2nd part (T₂) added to it 0.576 mmol/ ml of zinc sulphate. The 3rd part (T₃) added to it 5 mmol/ ml of cysteine. The 4th part (T₄) added to it 37.5 pg/ ml of PGF2 α and 5th part (T₅) added to it the combination of previous sub. The semen was cryopreserved in a liquid nitrogen and then after 30, 60 and 90 days. Seminal plasma was isolated by centrifugation with 3000 r/ min. for 10 min. the concentration of MDA in seminal plasma was measured by the measurement of Thiobarbutric acid (TBA) and Tri Chloro acetic acid (TCA) according to the method described by (10). The optical density measured with a spectrophotometer with a wave length of 532 nm and according to the following equation:

$$\text{MDA concentration= (mmol/ ml)} = \frac{\text{Reading of sample} - \text{Reading of control}}{\text{Absorption factor}} \times \text{Dilution factor}$$

Statistical analysis of data were done according to SAS (11) and Duncan multiple range test (12) using 1% level significant.

Results and discussion

The results showed in Table-1 that there was a significant decrease (P<0.01) in the concentration of MDA in the combination treatment (T₅) during the periods of freezing (30, 60 and 90) days, while the control (T₁) and the cysteine treatment (T₃) showed the highest value of MDA as compared with other treatment, the

PGF2 α and Zn. The decrease in values of MDA in combination treatment might be due to the synergistic effect of its content such as zinc acts as antioxidant (13,14), cysteine act as protectant to the sperms and nucleic acid (15). While PGF2 α provide the improvement of general characteristics of the sperms. The increase in MDA levels leads to decrease the quality of semen characteristics especially sperms motility (7). The MDA constitute the final end products of lipid peroxidation. It was formed as a results of conversion of

unsaturated fatty acids peroxide to MDA. It increase the formation of free radicals and oxidative stress (4, 5), that leads to damage of structure and functions of plasma membranes of bull sperm cells (6, 7).

Conclusion

It was concluded from this study the concentration of MDA in seminal plasma of Holstein bulls could be decrease in addition of Zn, cysteine and PGF2 α to cryopreserved semen *in vitro*.

Table (1) Effect of treatments on MDA level in seminal plasma of Holstein bulls (Mean \pm SE)

Treatments	Mean \pm SE (mmol/ ml)
Control (T ₁)	1.025 \pm 0.38 a
Zinc sulphate (T ₂)	0.867 \pm 0.12 a
Cysteine (T ₃)	1.06 \pm 0.12 a
PGF2 α (T ₄)	0.968 \pm 0.17 a
Combination (T ₅)	0.450 \pm 0.11 b

Different small superscripts showed significant difference (P<0.01)

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