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# Effect of Addition of Zn, Cysteine, PGF2α and their Combination on Holstein Bulls Cooled Semen *in vitro*

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**Abstract**

The study was conducted to investigate the effect of addition of zinc sulphate, cysteine, Prostaglandin F2α (PGF2α) and their combination to the diluted semen on semen characteristics of Holstein bulls after different periods of cooling. The study was conducted on Artificial insemination Center/ Directorate of Animal Resources/ Ministry of Agriculture at Abu-Graib at the west of Baghdad during the period of Aug. 2019 to the Dec. 2019. Seven Holstein bulls were used, Aged between 2.5- 3 years. Semen was collected via Artificial Vagina one ejaculate per a week for three months. Fresh semen was evaluated which were of bad grand pooled semen were divided into 5 parts. The first part ($T_1$) only diluted semen (Tris) serve as a control. The 2nd part ($T_2$) added 0.576 mmol/ ml of zinc sulphate to diluted semen. The 3rd part ($T_3$) added 5 mmol/ ml of cysteine to diluted semen. The 4th part ($T_4$) added 37.5 pg/ ml of PGF2α to diluted semen to diluted semen. The 5th part ($T_5$) added a combination of previous components to the diluted semen. Then the semen evaluated at 5C° after 24, 48 and 72 hrs. The results showed that the addition of zinc sulphate on cooled diluted semen have no significant difference between different periods in the percent of sperm individual motility. Addition of zinc sulphate and cysteine showed a significant increase ($P<0.05$) in the percent of sperm liveability. The addition of a combination (zn sulphate, cysteine and PGF2α) showed a significant decrease ($P<0.05$) in sperm abnormalities percent especially at 5C°. The results also showed the addition of zinc sulphate and PGF2α increase the percent of HOST (Hypo-osmotic swelling test) of spermatozoa at 48 hrs. after cooling. zinc sulphate addition showed a significant increase ($P<0.05$) at 5C° during as comported with other cooled period in the HOST. The addition of zinc sulphate showed a significant increase in cell membrane integrity at 5C° cooled semen. It was concluded that addition of antioxidant and hormone might improve semen quality of Holstein bulls.

**Keywards: Zn, Cysteine, PGF2α, cooled semen, in vitro, Holstein bulls.**

## تأثير إضافة الزنك، السستين، والبروستكلاندين وخليطها إلى السائل المنوي المبرد مختبرياً لثيران الهولشتاين

**الخلاصة**

أجريت هذه الدراسة لمعرفة مدى تأثير إضافة كل من الزنك، والسستين والبروستكلاندين كلٌ على حدة وخليطهما إلى السائل المنوي المخفف (مخفف Tris) في صفات السائل المنوي لثيران الهولشتاين بعد مدد مختلفة من التبريد. نفذت الدراسة في قسم التلقيح الاصطناعي، دائرة الثروة الحيوانية/ وزارة الزراعة في منطقة أبو غريب غرب بغداد للفترة من كانون الثاني 2019- وحتى شهر آب 2019. استخدم في هذه الدراسة سبعة ثيران هولشتاين تراوحت أعمارها من 2.5- 3 سنوات. جمع السائل المنوي بواسطة المهبل الاصطناعي وبواقع قذفة واحدة للثور أسبوعيا لمدة ثلاثة أشهر. أجريت فحوصات تقييم السائل المنوي الطازج ومن النوع الغير جيد، تم دمج القذفة للثيران جميعاً (Pooled semen) وتقسيمه على خمسة مجاميع مختلفة، أضيف إلى المجموعة الأولى (Control) مخفف ترس فقد عد بمثابة مجموعة سيطرة، في الوقت الذي تم إضافة (0.576 mmol/ ml) / من الزنك سلفيت إلى مخفف الترس والذي شكلت المجموعة الثانية من التجربة، بينما كانت المجموعة الثالثة تتكون من (5 mmol/ ml) من السستين المضاف إلى مخفف الترس 20 مل، أما المجموعة الرابعة فقد أضيف 37.5 pg/ m من هرمون البروستكلاندين إلى مخفف الترس 20 مل، أضيف إلى مخفف الترس توليفة من (0.576 mmol/ ml) من الزنك سلفيت و(5 mmol/ ml) من السستين و37.5 pg/ m من البروستكلاندين واعتبرت المجموعة الخامسة (مجموعة الخليط). تم دراسة تأثير الإضافات على صفات السائل المنوي خلال مدد التبريد عند درجة حرارة 5مْ (24، 48 و72) ساعة. أظهرت النتائج إن إضافة الزنك أظهرت فروق معنوية بين مدد التبريد الأربعة (5 مْ، 24، 48، 72) ساعة ($P<0.05$) للنسبة المئوية للحركة الفردية للنطف. حققت إضافة الزنك والسستين تفوقاً معنوياً ($P<0.05$) في النسبة المئوية للنطف الحية. كما حققت إضافة الخليط انخفاض معنوي ($P<0.05$) في نسبة النطف غير الطبيعة (المشوهة) وحصلت على اقل نسبة من النطف المشوهة عند فترة التبريد 5 مْ. وحققت إضافة كل من البروستكلاندين والزنك تفوقاً معنوياً ($P<0.05$) في النسبة المئوية لسلامة االغشاء البلازمي (HOST) في النطف عند فترة 48 ساعة من التبريد. كما حققت إضافة الزنك تفوقاً معنوياً ($P<0.05$) عند 5 مْ على باقي مدد التبريد في (HOST). تفوقت معاملة الزنك معنوياً ($P<0.05$) عند 5مْ على باقي المعاملات في النسبة المئوية لسلامة اكروسوم النطف (AI). نستنتج من الدراسة أن إضافة مضادات الأكسدة كالزنك، والسستين، وإضافة هرمون البروستكلاندين PGF2α قد حسن من نوعية السائل المنوى فى الثيران.

## Introduction

Artificial insemination plays an important role in genetic improvement in dairy bulls via which a single ejaculate from bull could inseminate many cows.

Cooling of semen storage facilitates semen transport for a distances and enables extension of superior genetic merit. Cooling have an exert effect on physiological as well as certain chemical stress on sperm cell (Chatterje et al., 2001). These stress may be induced by oxidative stress by free radical (Salvader et al., 2006). Sperm cells have a high content of unsaturated fatty acid but not an antioxidant in it's cell membrane, So the cell membranes is highly sensitive to the lipid peroxidation via free radical and $H_2O_2$ (Sinha et al., 1996).

Cellular damage resulted from oxidative stress due to reactive oxygen species (ROS) produced from cell components of semen during cooling, It may leads to decrease in motility and fertility during storage, while it may cause low temperature on structure of membrane of sperm destabilization (Mustafa and Necmettin, 2007).

The present study aimed to study the effect of addition of antioxidant such as zinc sulphate, cysteine and PGF2α on bull diluted and cooled semen.

## Materials and Methods

The current study was carried out to investigate the effect of addition of zinc sulphate, cysteine, Prostaglandin F2α and their combination to diluted semen on semen characteristics of Holstein bulls after different periods of cooling.

The study was conducted on seven Holstein bulls, Aged between 2.5- 3 years, presented at Artificial insemination centers/ Directorate of Animal Resources/ Ministry of Agriculture at Abu- Graib at the west of Baghdad during the period from December 2019 to August 2019.

Semen was collected by Artificial Vagina one ejaculate per a week for three months. Fresh semen was evaluated which were of bad grade. Pooled semen were divided into five parts. The 1st part ($T_1$) only diluted semen (Tris-based extender) serve as a control. The 2nd part ($T_2$) added 0.576 mmol/ ml of zinc sulphate to diluted semen. The 3rd part ($T_3$) added 5 mmol/ ml of cysteine to diluted semen. The 4th part ($T_4$) added 37.5 pg/ ml of PGF2α to diluted semen. The 5th part ($T_5$) added a combination of previous components to the diluted semen. Then the semen was evaluated on 5C° and after 24, 48, and 72 hrs. of cooling. The following parameters of semen were evaluated: Mass and Individual motility according to (5). Sperm abnormalities and a live spermatozoa according to (6). Measurements of sperm concentration with spectrophotometer according to the method of (7). Spermatozoal plasma membrane integrity percent were (HOST) calculated according to the method of (8). Acrosomal integrity percent using Gemsi stain according to the method of (9).

Statistical analysis were used according to SAS (10) and Duncan multiple range test(11).

## Results and Discussion

Table-1 showed semen parameters of fresh semen of Holstein bulls which is of bad quality. These parameters includes: ejaculate volume, mass motility, individual motility, liveability of spermatozoa, sperm concentration, sperm abnormalities, plasma membrane integrity and acrosomal integrity (AI).

Table-2 showed the effect of different treatment on different periods on individual motility of Holstein bulls semen. It has been shown that there was no significant difference between the five treatment during the periods of cooling at 5C°, 24, 48 and 72 hrs. on the percent of individual motility of spermatozoa, although there was mathematical difference between treatments. While there was a significant superiority during periods for the some treatment ($P<0.01$) at 5C° as compared with the periods of control, PGF2α, zinc sulphat and their combination. Cysteine treatment showed no differences during different periods of cooling at the same treatment. this superiority might be due to that the best temperature of preservation in cooling at 4-5 C° for several days (12, 13, 14).

These results a greed with (15) who claimed that the quality of semen decrease with the time of preservation till 48 hrs. according to the liveability of spermatozoa and the effect of preservation temperature.

Table- 3 showed the effect of different treatment on liveability of spermatozoa of Holstein bull after different periods of cooling. The results showed that the percent of spermatozoal liveability is highly significant ($P<0.05$) in zinc sulphate and cysteine treatments as compared

with control one. While there was no differences with PGF2α and combination treatments at 5C° of cooling. These differences has been explained by (16) who added 1 mg/ ml of zinc to the semen and observed no changes in the motility and the percent of forward movement as compared with other treatments. The results agreed with (17) who explained that zinc have the ability to protect sulfhydryl group (Sh) from oxidation enhance the synthesis of molecules rich with (Sh) which reduce glutathione and melatonin which acts as antioxidant. (18) reported that addition of zinc in a concentration of 0.8 mg/ ml to the bulls semen improve the concentration of spermatozoa and the acrosomal integrity of the sperms. The results of our study disagreed with (19) who observed that addition of high levels of zinc (50, 100 and 150) mmol/ ml to the diluted semen of Holstein bulls have no effect on morphology and motility of spermatozoa as compared with control one.

It has been reported that high concentration of zinc might decrease oxygen consumption in sperm cell respiration that affect motility. These variation might be due to differences in the concentration added by the workers (20).

Also the results agreed with (21) who explained that cysteine act on glutathione peroxidase that improve the content of spermatozoa from glutathione. It is also act on reduction of reactive oxygen species (ROS) and decrease lipid and nucleic acid oxidation that percent damage occurred to the head and protect the plasma membranes and mitochondria of sperm cells.

The results of cysteine disagreed with the (22) who reported the negative effect of addition of cysteine in a high concentration (12 and 15) mmol/ ml to the semen of Jersy bulls.

Table-4 showed the effect of different treatments on the percent of sperm cells abnormalities of Holstein bulls semen after different periods of cooling. It has been observed that there was a significant decrease ($P<0.05$) in the percent of sperm abnormalities at the combination treatment and PGF2α as compared with other treatments and different periods at 5C° and 24 hrs. This superiority of combination might be due to the synergistic effect between the components. The zinc showed a great effect on growth of the testes, spermatogenesis and the activity of spermatozoa, in addition to its action as antioxidant (17, 23). Cysteine acts as a reducing agent to ROS free radicals and decrease the lipid and nucleic acid oxidation with protection of head, plasma membrane and mitochondria of spermatozoa (24). (25) showed that addition of PGF2α in a concentration of 37.5 pg/ ml to the diluted and cooled semen improved semen characteristic of Holstein-Fresian bulls. Other workers showed a decrease in sperm abnormalities after addition of PGF2α to the semen of Frie-Raraan cross-Breed (26).

There was no information available on addition of this combination to the semen of bulls.

It has been observed that the best temperature of preservation for cooled semen at 4-5 C° (13, 14). The quality of semen reduced with the time of preservation till 48 hrs. according on the nature of diluent, temperature and the method of preparation of the diluent (15).

Table-5 showed the effect of different periods of cooling on hypo-osmotic swelling test (HOST) plasma membrane sperm cell integrity. The results showed that the zinc sulphate treatment differ significantly ($P<0.05$) from that of the combination while showed no differences with the control, PGF2α and cysteine treatment for the same periods of preservation at 5C°. This might be due to that the zinc protect sulfhydryl group protein from oxidation and acts to synthesized molecules rich in (Sh) that decrease the glutathione and melatonin which plays a role as antioxidant (17).

Our results a greed with the (27) who explained that addition of zinc leads to increase the integrity of spermatozoal cell membrane and reduce the damage of DNA of the cells. Similar results have been reported by (19) who observed that addition of zinc in concentration of (50, 100 and 150) mmol/ ml semen diluent of Holstein bulls increase the cell membrane integrity of the spermatozoa. Also (28) observed that addition of yeast enhanced with zinc and selenium to the ram ration leads to improvement of reproductive performance of the ram's (includes: ejaculate volume, individual motility, mass motility, liveability and concentration of spermatozoa). The results also showed there was a significant difference ($P<0.05$) in the characteristic of HOST between zinc and cysteine as compared with the PGF2α and the combination treatments during the period of preservation at 24 hrs.

The superiority of cysteine treatments might be

due to its action to protect the plasma membrane of bulls spermatozoa (29).

This is also in accordance with (30) who explained that addition of cysteine to Tris dilute of Holstein bulls semen leads to improvement of semen parameters.

The results also agreed with (25) on the effect of PGF2α and zinc significantly (P<0.05) during cooled preservation at 48 her.

Table-6 showed the effect of different treatment after different periods of cooling on acrosomal integrity (AI) of Holstein bulls semen.

The results showed that semen examined at 5C° the zinc treatments showed significant difference (P<0.05) in acrosomal integrity (AI) as compared with control, PGF2α and combination treatment. This might be due to the effect of zinc on lipid fluidity that affect stability of biological membrane and it is also participate and play a role in sperm capacitation and acrosomal reaction (31). These results agreed with (32) who reported that zinc additives protect sperm cells from bacterial infection and prevent chromosomal damage. Similar observations have been made by (18).

## Conclsion

It was concluded from this study that addition, of zinc sulphate, cysteine, PGF2α to the Holstein bulls semen improve the characteristics of semen parameters.

Table (1) semen characteristics of Holstein bulls (Mean ± SE)

| Characteristics | Mean ± SE |
|---|---|
| Volume (ml) | 6.33 ± 0.33 |
| Mass motility (%) | 35.83 ± 1.72 |
| Individual motility (%) | 42.50 ± 1.68 |
| liveability of spermatozoa (%) | 82.58 ± 1.64 |
| Sperm concentration (million/ ml) | 1403.48 ± 122.27 |
| Sperm abnormalities (%) | 9.04 ± 1.06 |
| Plasma membrane integrity (HOST) (mOsm/L) | 70.91 ± 1.90 |
| Acrosomal integrity (AI) (%) | 75.50 ± 1.58 |

Table (2) Effect of treatment and time on the individual motility of sperm in the ejaculate of Holstein bulls after different periods of cooling (Mean ± SE)

| Treatment | 5 C° | 24 hrs. | 48 hrs. | 72 hrs. | Level of significance |
|---|---|---|---|---|---|
| Control | 41.67 ± 2.24 a A | 29.44 ± 4.36 a B | 28.00 ± 3.88 a B | 27.85 ± 4.47 a B | * |
| Zinc sulphate | 44.09 ± 3.55 a A | 30.00 ± 4.56 a B | 30.00 ± 4.53 a B | 27.50 ± 5.17 a B | * |
| Cysteine | 41.00 ± 4.00 a A | 28.89 ± 5.82 a A | 27.89 ± 4.98 a A | 27.85 ± 5.65 a A | N.S |
| PGF2α | 50.00 ± 2.95 a A | 36.11 ± 4.84 a B | 32.00 ± 4.29 a B | 31.87 ± 4.52 a B | * |
| Combination | 50.00 ± 3.01 a A | 35.00 ± 4.88 a B | 35.00 ± 4.82 a B | 33.89 ± 5.45 a B | * |
| Level of significance | N.S | N.S | N.S | N.S | -- |

*The different capital letters refer to significant differences between different periods (raw) at (P≤0.01)
NS= Non-significant.

Table (3) Effect of treatment and time on the liveability of spermatozoa in the semen of Holstein bulls after different periods of cooling (Mean ± SE)

| Treatment | 5 C° | 24 hrs. | 48 hrs. | 72 hrs. | Level of significance |
|---|---|---|---|---|---|
| **Control** | 74.41 ± 1.67 a A | 72.89 ± 5.00 a A | 71.10 ± 3.79 a A | 68.57 ± 3.08 a A | N.S |
| **Zinc sulphate** | 80.90 ± 1.53 a A | 79.00 ± 1.02 a A | 77.20 ± 1.76 a A | 77.00 ± 2.87 a A | N.S |
| **Cysteine** | 77.50 ± 1.40 a A | 75.43 ± 1.79 a A | 74.70 ± 2.88 b A | 72.89 ± 2.53 a A | N.S |
| **PGF2α** | 76.41 ± 1.37 ab A | 74.00 ± 2.51 a A | 72.10 ± 2.33 a A | 72.00 ± 2.44 a A | N.S |
| **Combination** | 76.91 ± 1.75 ab A | 76.00 ± 1.83 a A | 73.25 ± 3.13 a A | 70.78 ± 2.80 a A | N.S |
| **Level of significance** | * | N.S | * | N.S | -- |

*The different small letters refer to significant differences between different treatment groups (column) at (P≤0.05)
NS= Non-significant.

Table (4) Effect of treatment and time on the Sperm abnormalities in the semen of Holstein bulls after different periods of cooling (Mean ± SE)

| Treatment | 5 C° | 24 hrs. | 48 hrs. | 72 hrs. | Level of significance |
|---|---|---|---|---|---|
| **Control** | 9.83 ± 0.88 a C | 14.33 ± 1.05 ab B | 15.50 ± 1.53 a AB | 19.42 ± 2.44 a A | ** |
| **Zinc sulphate** | 9.00 ± 0.82 a C | 14.89 ± 0.90 ab B | 16.50 ± 1.34 a AB | 19.12 ± 2.07 a A | ** |
| **Cysteine** | 9.10 ± 1.14 a B | 16.62 ± 1.95 a A | 17.22 ± 1.81 a A | 18.57 ± 2.09 a A | ** |
| **PGF2α** | 9.12 ± 0.41 a C | 12.67 ± 1.06 ab B | 16.70 ± 1.32 a A | 16.87 ± 1.95 a A | ** |
| **Combination** | 6.16 ± 0.93 b B | 13.89 ± 1.33 ab A | 15.10 ± 1.86 a A | 16.00 ± 2.04 a A | ** |
| **Level of significance** | * | * | N.S | N.S | -- |

*The different small letters refer to significant differences between different treatment groups (column) at (P≤0.05)
**The different capital letters refer to significant differences between different periods (raw) at (P≤0.01)
NS= Non-significant.

Table (5) Effect of treatment and time on the (HOST) in the semen of Holstein bulls after different periods of cooling (Mean ± SE)

| Treatment | 5 C° | 24 hrs. | 48 hrs. | 72 hrs. | Level of significance |
|---|---|---|---|---|---|
| Control | 68.50 ± 1.29 ab A | 68.00 ± 1.47 ab A | 67.00 ± 1.82 b A | 65.71 ± 2.24 b A | N.S |
| Zinc sulphate | 73.33 ± 2.04 a A | 73.30 ± 1.09 a A | 73.29 ± 1.55 a A | 71.62 ± 2.12 a AB | ** |
| Cysteine | 71.70 ± 1.56 ab A | 69.43 ± 0.99 a AB | 68.62 ± 1.95 ab AB | 66.44 ± 1.16 b B | ** |
| PGF2α | 68.83 ± 1.83 ab A | 66.80 ± 2.61 b A | 66.75 ± 2.69 a A | 64.87 ± 1.51 b A | N.S |
| Combination | 67.67 ± 2.09 b A | 67.67 ± 1.85 b A | 66.33 ± 2.71 b A | 66.25 ± 2.65 a A | N.S |
| Level of significance | * | * | * | * | -- |

*The different small letters refer to significant differences between different treatment groups (column) at (P≤0.05)
**The different capital letters refer to significant differences between different periods (raw) at (P≤0.01)
NS= Non-significant.

Table (6) Effect of treatment and time on the Acrosomal integrity (AI) in the semen of Holstein bulls after different periods of cooling (Mean ± SE)

| Treatment | 5 C° | 24 hrs. | 48 hrs. | 72 hrs. | Level of significance |
|---|---|---|---|---|---|
| Control | 69.83 ± 1.58 b A | 71.22 ± 2.68 ab A | 68.50 ± 2.17 ab A | 68.00 ± 1.94 a A | N.S |
| Zinc sulphate | 74.36 ± 1.27 a A | 75.55 ± 2.29 a A | 73.00 ± 1.54 a A | 72.37 ± 2.27 a A | N.S |
| Cysteine | 70.70 ± 1.11 ab A | 70.87 ± 1.84 ab A | 70.71 ± 1.73 a A | 68.78 ± 1.40 a A | N.S |
| PGF2α | 70.08 ± 1.04 b A | 69.67 ± 2.14 ab A | 69.50 ± 2.25 a A | 67.20 ± 1.59 ab A | N.S |
| Combination | 69.50 ± 1.70 b A | 70.50 ± 1.74 ab A | 66.67 ± 2.06 b A | 66.25 ± 2.49 b A | N.S |
| Level of significance | * | * | * | * | -- |

*The different small letters refer to significant differences between different treatment groups (column) at (P≤0.05)
NS= Non-significant.

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