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INFLUENCE OF LIPID PEROXIDATION INHIBITORS ON PRESERVABILITY OF BALADI BULL SEMEN I- DOSE-RESPONSE STUDY

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ABSTRACT

Fortification of citrate-based extenders with lipid peroxidation inhibitors significantly ($P < 0.05$) improved the preservability of chilled bull semen. The superior values of sperm motility and viability were recorded for 2.80mg pentoxifylline /ml; 1.00 mg EDTA / ml; 2.00mg sodium thiosulfate / ml; 0.10mg ascorbic acid /ml; 1.00mg methionine / ml; 0.005mg epinephrine /ml; 2.00mg mannitol /ml; 0.43mg sodium benzoate / ml and 0.02mg 17b- Estradiol /ml.

INTRODUCTION

The ultimate merit of conservation of bovine semen in a liquid state is that far fewer spermatozoa are required per insemination doses to achieve the same level of fertility compared with frozen semen **(Yoshida, 2000)**. Nevertheless, the short fertile life of unfrozen semen makes it a less flexible option **(Krzyzosiak et al., 2000)**.

Throughout the last two decades, a considerable amount of evidences has accumulated to suggest that excessive generation of reactive oxygen species (ROS) during processing and storage of bull semen in egg yolk-based extenders procured a dramatic loss in the motility and fertilizing ability of spermatozoa owing to the initiation of a lipid peroxidation cascade in sperm membranes **(Vishwanath and Shannon, 1997, 2000)**. Likewise, the free radical theory of sperm aging postulated that in the early stages of spermatozoal senescence, lipid peroxidation triggered degenerative changes in the inner mitochondrial membrane and nuclear membranes of chilled-stored spermatozoa **(Vishwanath and Shannon, 1997; Cerolini et al., 2000)**.

The current study was, therefore, performed to investigate whether the motility of chilled-stored bull spermatozoa could be maintained for extended periods by supplementation of semen extenders with some inhibitors of sperm lipid peroxidation. If so, what is the appropriate concentration of each inhibitor that could attain the maximum values of sperm motility and viability.

MATERIALS AND METHODS

Chemical reagents and semen extenders

Unless otherwise stated, all chemicals used in this study were of the highest available grade and purchased from Sigma-Aldrich Co., Deisenhofen, Germany. For short-term preservation of bull semen, egg yolk-citrate diluent **(Dhami et al., 1993)** was used. It was composed of 73.00ml sodium citrate buffer (prepared by dissolving 2.90g trisodium citrate dihydrate in 100.00ml glass-distilled water), 20.00ml fresh chicken egg yolk and 7.00ml glycerol. Antibiotics were added to the diluent at concentrations of 1000.00IU/ml penicillin G sodium and 1.00mg/ml streptomycin sulfate.

Semen collection and processing

Semen samples were collected twice a week by means of an artificial vagina from four Baladi bulls (5-7 years old) belonging to a private farm at **El-Manzala**, Dakahlia, Egypt. Within 5 minutes following collection, semen samples were transferred to the laboratory and kept in a water bath at 30°C for initial evaluations including semen volume, sperm concentration, mass movement, individual motility, live spermatozoa and sperm morphological abnormalities **(Khalifa, 2001)**. Only ejaculates of at least 65% initial motility and $700x10^6$ sperm cells/ml were used in a series of nine in vitro experiments. In each experiment, 20 ejaculates from bulls (5 ejaculates/bull) were split and diluted (1:4) at 30°C with semen diluents supplemented with or without the following concentrations of sperm lipid peroxidation inhibitors: -

- **Experiment 1:** 1.40, 2.80 and 4.20mg/ml (w/v) pentoxifylline (white powder).
- **Experiment 2:** 1.00, 2.00 and 3.00mg/ml (w/v) EDTA (Free acid, anhydrous).
- **Experiment 3:** 1.00, 2.00 and 3.00 mg/ml (w/v) sodium thiosulfate pentahydrate.
- **Experiment 4:** 0.10, 0.50, 1.00 and 1.50mg/ ml (w/v) L-ascorbic acid (Free acid, fine crystals).
- **Experiment 5:** 1.00, 2.00 and 3.00mg/ml (w/v) DL-methionine (DL-2-Amino-4-methylthiol-butanoic acid, crystalline, BDH Chemical Co. England).
- **Experiment 6:** 0.005, 0.010 and 0.015 mg/ml (w/v) (±)- epinephrine hydrochloride.
- **Experiment 7:** 1.00, 2.00 and 3.00 mg/ml (w/v) D-mannitol (Crystalline).
- **Experiment 8:** 0.43, 0.86 and 1.30mg/ml (w/v) sodium benzoate.
- **Experiment 9:** 0.01, 0.02 and 0.04 mg/ml (w/v) 17β-Estradiol. Since Estradiol is a fat soluble compound, therefore, it has been decided to dissolve these concentrations in a suitable fat solvent (dimethyl sulfoxide: DMSO) that has not any detrimental effect on the viability of

bull spermatozoa at a concentration (v/v) of 1.00% **(Snedeker and Gaunya, 1970)**. Also, because DMSO has been shown to have an inhibitory effect on sperm lipid peroxidation **(El-Saidy et al., 2000)**, a second control diluent containing 1.00% (v/v) DMSO was used as a semen treatment in this experiment.

Within 10 minutes after dilution, the extended semen was placed in the refrigerator and incubated at 5°C for 168 hours. The minimum number of progressively motile spermatozoa per ml of diluted semen was $90x10^6$. Sperm progressive motility was subjectively assessed after dilution, as well as after 6, 24, 48, 72, 96, 120, 144 and 168 hours of incubation period using a phase-contrast microscope (400X) equipped with a thermal stage at 37°C. The viability index of incubated semen was calculated according to **Milovanov (1962)**.

Statistical analyses

Using the general models procedures of the Statistical Analysis Systems **(SAS, 1990)**, data obtained from each experiment were subjected to analysis of variance (one way ANOVA) to clarify the influence of semen treatments on sperm motility and viability indices. Treated means were compared by the least significant difference test (LSD) at 5% level of probability.

RESULTS

Before processing of bull semen for hypothermic preservation, examination of freshly ejaculated semen samples, throughout the present investigation, revealed that the overall mean percentages of progressive motile sperm, live spermatozoa and total sperm abnormalities were 71.00%, 81.20% and 13.86%, respectively.

As depicted in table 1, fortification of semen extenders with 2.80mg/ ml pentoxifylline resulted in a significant ($P<0.05$) improvement in sperm motility after incubation of chilled bull semen for 72 and 168 hours. Also, at the aforementioned concentration of pentoxifylline, the maximum value (123.83 ± 3.45) of viability index was obtained.

Table 2 outlines the effect of three levels of EDTA on preservability of bull semen. It was evident that supplementation of citrate-based diluents with 1.00mg/ml EDTA induced a significant ($P<0.05$) augmentation in the viability index and sperm motility percentages at all incubation periods. On the contrary, inclusion of EDTA in semen extenders at a concentration of 3.00mg/ml significantly ($P<0.05$) minimized the motility and viability indices of stored spermatozoa.

Data regarding the influence of sodium thiosulfate on the quality of preserved semen are clarified in table 3. In vitro provision of semen extenders with 2.00 or 3.00mg/ml sodium thiosulfate significantly ($P < 0.05$) exerted an explicit increase in sperm motility after dilution as well as after

6 hours of incubation at 5°C. The superior value (95.70±5.04) of viability index was achieved after treatment of bull spermatozoa with sodium thiosulfate at a concentration of 2.00mg/ml.

Table 4 displays the outcomes of in vitro exposure of bull semen to different concentrations of ascorbic acid. It was clear that ascorbic acid at a concentration of 0.10mg/ml had a significant ($P<0.05$) amendatory effect on storageability of semen. The highest percentages of sperm motility after 72 hours (64.50±2.17%) and 168 hours (44.50±3.37%) of incubation period, as well as the greatest value (106.44±3.30) of viability index were attained by the above-mentioned level of ascorbic acid.

Table 5 points out the impact of methionine on the quality of preserved semen. Definitely, prestorage treatment of bull spermatozoa with 1.00mg/ml methionine significantly ($P< 0.05$) provoked a detectable enhancement in their motility after 168 hours of incubation period. Concomitantly, at the same concentration of methionine, the maximum value (120.03±2.85) of viability index was recorded.

Table 6 illustrates the influence of epinephrine on the livability of chilled-stored semen. As a rule, the motility percentages and viability indices of spermatozoa supplemented with 0.005mg/ml epinephrine were significantly ($P< 0.05$) higher than that of spermatozoa supplemented with or without 0.010 and 0.015mg/ml epinephrine. At 168 hours of incubation period, analysis of variance could not detect any significant variation among the motility percentages of spermatozoa treated with 0.005 and 0.010mg/ml epinephrine.

With respect to the effect of mannitol on preservability of bull semen, table 7 demonstrates a pronounced ($P<0.05$) improvement in the viability indices of stored spermatozoa after supplementation of diluents with 1.00, 2.00 or 3.00mg/ml. The maximum percentages of sperm motility after 6 hours (81.50±0.76%), 72 hours (77.00±1.11%) and 168 hours (53.50±3.95%) of incubation period were observed after treatment of semen with mannitol at a concentration of 2.00mg/ml.

Table 8 explicates the effect of various doses of sodium benzoate on the quality of stored bull semen. Broadly, the mean values of viability indices and sperm motility (at 72 and 168 hours) of semen exposed to 0.43mg/ml were significantly ($P<0.05$) higher not only than that of control but also than that of semen exposed to 0.86 and 1.30mg/ml.

As indicated in table 9, in vitro fortification of bull semen extenders with increasing concentrations (0.01, 0.02 and 0.04mg/ml) of 17β-Estradiol resulted in a significant ($P<0.05$) increment in the motility and viability indices of incubated spermatozoa. The maximum percentages of sperm motility after dilution (80.00±1.07%), 6 hours (78.61±0.97%), 72 hours (73.06±0.72%) and 168 hours (45.28±2.51%) of incubation period as well as the superior value (120.48±1.70) of

viability index were detected after treatment of semen with Estradiol at a concentration of 0.02mg/ml.

DISCUSSION

Movement is the major energy-demanding process in spermatozoa that has been considered a prerequisite criterion in the assessment of how many sperm cells are functionally competent to carry out the fertilization process **(Gagnon and Lamirande, 1995)**.

In spite of the existence of antioxidant defense mechanisms within mammalian sperm, the flux of local oxidant generation in consequence of low-temperature manipulation of semen may frequently overwhelm these defenses, resulting in the occurrence of sperm lipid peroxidation **(Erokhin et al., 1996)**. Loss of flagellar beat frequency, inactivation of sperm metabolic enzymes, reduction of intracellular adenosine triphosphate (ATP) levels and attenuation of cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of axoneme proteins have all been associated with the accumulation of lipid peroxidation products in sperm membranes **(Gagnon, 1997)**.

Supporting the above-mentioned concept, our data clearly highlight the pivotal role of lipid peroxidation inhibitors in maintaining the longevity of chilled-stored bull semen.

Supplementation of semen extenders with pentoxifylline at a concentration of 2.80mg/ml resulted in a marked improvement in sperm motility and viability. These results did not coincide with findings of **Gotz (1995)** who recorded the inability of pentoxifylline (2.00mg/ml) to improve bull sperm motility. However, in another study, **Vega et al. (1997)** found that postthaw supplementation of cryopreserved bull semen with 6.00 or 10.00 mM pentoxifylline could prolong the viability of spermatozoa. Recently, in an endeavor to find out the influence of pentoxifylline on preservability of semen, **Khalifa (2001)** detected a significant augmentation in sperm motility and viability after its addition to ram and buffalo semen extenders at concentrations of 0.50 and 5.00mM, respectively. The favourable influence of pentoxifylline on sperm motility was ascribed to its ability to safeguard sperm membranes from the peroxidative damage of reactive oxygen species **(Sharma and Agarwal, 1997; El-Sheltawi et al., 2002)**. Furthermore, it has been suggested that pentoxifylline was capable of increasing the intracellular levels of cAMP, a molecule involved in regulation of sperm kinematic activity **(Ramesha et al., 2000)**.

Fortification of bull semen extenders with a low level (1.00mg/ml) of EDTA caused a significant increase in the motility and viability of chilled-stored spermatozoa. In agreement with the present data, **Dhami et al. (1993, 1995)** observed that inclusion of 0.10% EDTA in citrate-egg

yolk- glycerol diluents had a significant beneficial effect on the motility, viability and fertility of frozen-thawed and liquid (5°C) semen of Friesian bulls. The amendatory effect of EDTA on preservability of semen was attributed to its tendency to alleviate the peroxidative damage incurred by releasing of amino acid oxidase from dead spermatozoa **(Shannon and Curson, 1972, 1982).**

Paradoxically, the current study revealed a dramatic decrease in sperm motility and viability after inclusion of a high level (3.00mg/ml) of EDTA in semen diluents. In accord with our observation, **Vishwanath et al. (1992)** in their study on the effect of chelating agents and free radical scavengers on survival of bull spermatozoa at ambient temperature, noticed that diluents fortified with 1.00 to 10.00 mM EDTA were significantly poorer than the control in maintaining sperm motility. As a matter of fact, the involvement of EDTA in cessation of sperm metabolic activity is beyond doubt because at a high concentration EDTA could inhibit the activity of spermatozoal adenosine triphosphatase **(Mann and Lutwak-Mann, 1981)**, stimulate sperm lipid peroxidation **(Aitken et al., 1993)**, and minimize the intracellular level of calcium ions **(Centola, 1998)**.

Concerning the impact of sulfur- containing compounds on storageability of bull semen, the present work recorded a remarkable increment in sperm motility after addition of sodium thiosulfate (2.00mg/ml) or methionine (1.00 mg/ml) to citrate-based extenders.

Our data did potentiate the findings of **Nauk and Gus'kov (1983) and Nauk (1988)** who justified that inclusion of sodium thiosulfate in bull semen extenders resulted in a reduction in the rate of sperm lipid peroxidation and a subsequent enhancement in sperm motility and survivability. However, **Moroz et al. (1990) and Lapointe and Sirard (1998)** ruled out the ability of methionine to improve bull sperm motility.

It is worthy to note under our experimental conditions that the favourable influence of methionine on sperm motility may be relied on its capability to maintain a high level of alpha- tocopherol in seminal plasma and spermatozoa **(Kaludin and Dimitrova, 1986)**. Alpha-tocopherol was detected to be a potent chain-breaking antioxidant that could inhibit the propagation of lipid peroxidation in the sperm membranes **(Beconi et al., 1993)**.

As regards the effect of hydroxyl radical scavengers on longevity of bull semen, the current investigation detected a pronounced augmentation in sperm motility and viability after in vitro provision of citrate-based diluents with ascorbic acid (0.10 mg/ml) or mannitol (2.00 mg/ml) or sodium benzoate (0.43mg/ml). Nearly similar effects were obtained by **Beconi et al. (1993)** who observed that fortification of bull semen extenders with 5mM sodium ascorbate induced a significant improvement in sperm motility. Moreover, **Khalifa (2001)** found that supplementation of ram semen extenders with 0.10% mannitol or 3.00 mM sodium benzoate resulted in a significant

increase in the viability of chilled - stored spermatozoa. The beneficial influence of ascorbic acid or mannitol or sodium benzoate in semen diluents may be resided in its ability to inhibit the peroxidation of polyunsaturated fatty acids in sperm membranes by neutralization of hydroxyl radicals, protection of intracellular superoxide dismutase activity, regeneration of the antioxidant activity of the oxidized form of alpha-tocopherol in sperm membranes and reducing the delcterious effects of hydrogen peroxide in stored sperm cells **(Bize et al., 1991; Beconi et al., 1993; Tonetti et al ., 1993; Dalvit et al. , 1998)**.

Epinephrine (adrenaline) is a normal constituent of bovine seminal plasma **(Mann and Lutwak-Mann, 1981)** that, immediately after ejaculation, binds to alpha-adrenergic receptors on sperm plasma membrane and mediates several cellular functions including an increase in the influx of free calcium ions into sperm cells **(Meizel, 1985)** and stimulation of sperm motility **(Nelson and Cariello, 1989)** as well as sperm transport in the female reproductive tract **(Gallagher and Senger, 1989)**.

Hand in hand with the aforementioned findings, our data evidently revealed that treatment of bull semen with a low concentration (0.005mg/ml) of epinephrine induced a significant increase in sperm motility not only immediately after dilution but also after extended periods of incubation at 5°C. It seemed that epinephrine at this level might be able to maintain the viability of bull spermatozoa by inhibiting the rate of lipid peroxidation **(Alvarez and Storey, 1983)**.

On the contrary, the present investigation indicated that exposure of bull semen to a high dose (0.015mg/ml) of epinephrine caused a significant decrease in sperm motility after 72 hours of incubation period. Concomitantly, **Mann (1964)** reported that when adrenaline was added to bull semen in relatively high concentrations (10.00-100.00µg/ml), the aerobic, but not the anaerobic, fructolysis was gradually inhibited due to aerobic formation of adrenochrome by catalytic action of the cytochrome system of spermatozoa upon adrenaline or due to formation of hydrogen peroxide by the catalytic action of seminal plasma monoamine oxidase (adrenaline oxidase) on adrenaline.

17b- Estradiol represents one of the normal constituents of mammalian seminal plasma **(Raeside and Christie, 1997)** that, upon ejaculation, binds to sperm plasma membrane and regulates spermatozoal functions **(Luconi et al., 1999)**. There have been claims that in addition to be metabolized by sperm cells **(Seamark and White, 1964)**, estradiol at a low concentration (2.00µg/ml) is able to activate sperm adenylate cyclase **(Cheng and Boettcher, 1982)** and may be involved in the stimulation of sperm transport in the female reproductive tract **(Orihuela et al., 1999)**.

Interestingly, a potent antioxidant action of estrogens, which exceeds that of alpha-

tocopherol, has only recently been realized **(Behl et al., 1995; Murdoch, 1998)**. Estradiol at a concentration of 320.00 pg/ml can safeguard the living cells from hydrogen peroxide- induced cell death **(Wiseman et al., 1993; Lund et al., 1999, Wise et al., 1999)**.

In view of the above-stated disputations, as well as in agreement with the findings of the previous authors **(Hicks et al., 1972; Daader and El-Keraby, 1982; Marinov et al., 1983; Idaomar et al., 1989)**, our investigation recorded the maximum improvement in sperm motility and viability after supplementation of semen diluents with estradiol at a concentration of 0.02mg/ml. It seems that the tendency of Estradiol to mediate hydrogen- transfer process **(Mann, 1964)** may be responsible for the elimination of hydrogen peroxide from the sperm cells, thereby inhibiting peroxidation of phospholipids in sperm membranes.

In conclusion, supplementation of citrate-based extenders with lipid peroxidation inhibitors could maintain the motility of chilled bull semen up to 168 hours. Experiments are in progress to compare between these inhibitors in an attempt to select the best one.

Table 1: Effect of pentoxifylline on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Pentoxifylline concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	73.00 ± 2.49	72.00±2.71	66.50±1.50^{a}	40.00±3.65^{a}	106.80±4.18
1.40	75.50 ± 3.02	77.50±3.27	71.50±1.80^{b}	50.50±4.11^{ab}	118.41±4.92
2.80	75.50 ± 2.83	77.50±2.27	72.50±2.01^{b}	55.00±2.58^{b}	123.83±3.45
4.20	77.50 ± 2.50	80.50±2.17	73.00±1.86^{b}	48.50±4.66^{ab}	120.63±3.71

Means with different superscripts in the same column are significantly different (P < 0.05)

Table 2: Effect of EDTA on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

EDTA concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	76.50 ± 1.68^{a}	76.00±1.63^{a}	67.00±1.86^{a}	38.00±4.16^{a}	108.81±4.03^{a}
1.00	82.50 ± 1.71^{b}	79.50±1.17^{b}	73.50±1.07^{b}	47.00±3.51^{b}	120.93±2.34^{b}
2.00	79.50 ± 1.17^{ab}	79.00±1.25^{ab}	63.00±2.91^{a}	38.00±3.96^{a}	103.91±5.78^{a}
3.00	75.50 ± 1.89^{a}	68.00±2.26^{c}	32.00±3.89^{c}	8.50±1.07^{c}	53.22±3.80^{c}

Means with different superscripts in the same column are significantly different (P < 0.05)

Table 3: Effect of sodium thiosulfate on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Sodium thiosulfate concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	69.50 ± 0.90^{a}	67.50±1.12^{a}	58.50±3.25	14.00±3.14	82.59±3.22^{a}
1.00	71.00 ± 1.25^{ab}	70.50±1.38^{ab}	63.50±2.99	24.00±4.52	93.96±4.43^{b}
2.00	73.00 ± 1.33^{bc}	73.00±1.33^{b}	65.50±2.63	19.50±5.50	95.70±5.04^{b}
3.00	75.00 ± 1.29^{c}	73.50±1.07^{b}	62.50±2.39	13.00±2.13	88.99±3.53^{ab}

Means with different superscripts in the same column are significantly different (P < 0.05)

Table 4: Effect of ascorbic acid on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Ascorbic acid concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	73.00 ± 1.33	71.00±1.00	55.50±2.41	33.50±3.17^{ac}	94.35±2.15^{ac}
0.10	75.00 ± 1.49	72.00±1.33	64.50±2.17	44.50±3.37^{b}	106.44±3.30^{b}
0.50	72.50 ± 1.71	72.00±1.70	60.00±5.11	36.00±3.71^{a}	99.41±5.37^{ab}
1.00	73.00± 2.00	72.00± 2.26	54.50± 5.84	29.50± 3.37^{ac}	94.55± 6.74^{ac}
1.50	74.50± 1.17	74.00±1.25	55.00±6.06	25.50±6.47^{c}	87.09±8.86^{c}

Means with different superscripts in the same column are significantly different (P < 0.05).

Table 5: Effect of methionine on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Methionine concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	74.00 ± 1.94	74.00±1.94	64.00±2.08	45.00±2.01^{a}	105.38±2.11^{a}
1.00	77.00 ± 2.00	77.00±2.00	71.50±2.11	52.00±1.12^{b}	120.03±2.85^{b}
2.00	78.50 ± 2.69	78.50±2.69	68.50±3.41	46.50±2.80^{ab}	115.37±3.66^{ab}
3.00	79.00± 2.87	79.00± 2.87	74.00± 2.77	46.00± 2.52^{ab}	119.25± 4.61^{ab}

Means with different superscripts in the same column are significantly different (P < 0.05).

Table 6: Effect of epinephrine on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Epinephrine concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	74.00 ± 1.25^{a}	71.50±1.68^{a}	67.00±1.33^{a}	31.50±3.66^{a}	102.20±3.88^{ac}
0.005	78.00 ± 1.11^{b}	78.00±1.11^{b}	77.00±0.82^{b}	49.50±5.02^{b}	124.67±3.37^{b}
0.010	73.50 ± 1.30^{a}	70.50±2.03^{a}	67.50±2.61^{a}	45.50±4.56^{bc}	111.89±5.01^{a}
0.015	73.50± 1.68^{a}	68.00±3.18^{a}	58.50± 3.17^{c}	37.50± 4.10^{ac}	98.13± 5.95^{c}

Means with different superscripts in the same column are significantly different (P< 0.05).

Table 7: Effect of mannitol on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Mannitol concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	78.00 ± 0.82	77.50±0.83^{a}	70.00±1.05^{a}	36.00±2.21^{a}	112.11±1.95^{a}
1.00	79.00 ± 1.00	78.50±1.07^{ab}	75.50±1.57^{b}	47.50±3.44^{b}	122.99±2.23^{bc}
2.00	81.50 ± 0.76	81.50±0.76^{b}	77.00±1.11^{b}	53.50±3.95^{b}	128.87±2.50^{c}
3.00	79.00± 1.25	76.00± 1.80^{a}	71.00± 0.67^{a}	50.50± 3.29^{b}	118.70± 2.11^{b}

Means with different superscripts in the same column are significantly different ($P < 0.05$).

Table 8: Effect of sodium benzoate on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Sodium benzoate concentrations (mg/ml)	After dilution	Incubation periods (hours)			Viability indices
		6	72	168	
Control	76.00 ± 1.00	75.00±1.05	67.00±1.86^{a}	43.50±3.38^{a}	110.99±4.42^{a}
0.43	79.50 ± 1.38	79.50±1.38	75.50±1.74^{b}	57.50±4.49^{b}	128.96±3.43^{b}
0.86	76.50 ± 1.38	75.50±2.03	68.00±2.00^{a}	41.50±4.22^{a}	110.94±4.47^{a}
1.30	77.00± 1.11	76.50±1.30	69.50± 1.57^{a}	43.00±4.36^{a}	113.99± 3.65^{a}

Means with different superscripts in the same column are significantly different ($P < 0.05$).

Table 9: Effect of 17β-Estradiol on motility (%) and viability of chilled-stored bull spermatozoa (Means ± SE).

Semen treatments		After dilution	Incubation periods (hours)			Viability indices
			6	72	168	
Control		74.72± 0.75^{a}	71.39±0.97^{a}	60.56±1.39^{a}	28.61±2.85^{a}	94.38±2.59^{a}
1.00 % DMSO		74.44 ± 0.38^{a}	71.94±0.72^{a}	61.94±0.72^{a}	30.00±1.81^{a}	98.13±1.50^{a}
Estradiol concentrations (mg/ml) dissolved in 1.00% DMSO	0.01	78.89 ± 0.76^{bc}	76.94±0.92^{bc}	70.83±0.93^{bc}	41.67±2.97^{b}	114.24±2.12^{b}
	0.02	80.00± 1.07^{c}	78.61±0.97^{c}	73.06± 0.72^{c}	45.28± 2.51^{b}	120.48± 1.70^{b}
	0.04	77.22± 1.01^{b}	75.28±1.11^{b}	68.89±0.95^{b}	43.89±3.12^{b}	117.64±4.04^{b}

Means with different superscripts in the same column are significantly different ($P < 0.05$).

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الملخص العربى

تأثير مثبطات تأكسد الدهون على حفظ السائل المنوى لطلائق الأبقار البلدية.

١- إختيار أفضل جرعة من مثبطات التأكسد

المشتركون فى البحث

طارق عبدالوهاب عبدالمحسن خليفه - سامى معوض زعبل

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أجريت تسعة تجارب معملية على السائل المنوى لأربعة طلائق أبقار بلدية تتراوح أعمارها من ٥ إلى ٧ سنوات، وكان الهدف هو دراسة تأثير إضافة جرعات مختلفة من بعض مثبطات تأكسد الدهون إلى مخففات السائل المنوى على حركة وحيوية الحيوانات المنوية المحفوظة عند درجة حرارة ٥° م ولمدة ١٦٨ ساعة.

ولقد أظهرت النتائج أن أعلى تحسن فى نسبة الحركة الأمامية للحيامن وكذلك فى قيم دليل الحيوية قد نتج من إضافة البنتوكسفللين عند تركيز ٢ر٨٠ ملجم/مللى، والإدتا عند تركيز ١ر٠٠ ملجم/مللى، وثيوسلفات الصوديوم عند تركيز ٢ر٠٠ ملجم/مللى، وحامض الأسكوربك عند تركيز ١ر٠ ملجم/مللى، والمثيونين عند تركيز ١ر٠٠ ملجم/مللى، والإبنفرين عند تركيز ٠٠٥ر٠ ملجم/مللى، والمانيتول عند تركيز ٢ر٠٠ ملجم/مللى، وبنزوات الصوديوم عند تركيز ٠٤٣ر٠ ملجم/مللى، والإستراديول عند تركيز ٠٢ر٠ ملجم/مللى.