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Antioxidative Effect of Addiing Taurine and Leptin to Tris-Extenders on Freezing and Fertilizing Abilities of Buffalo-Semen

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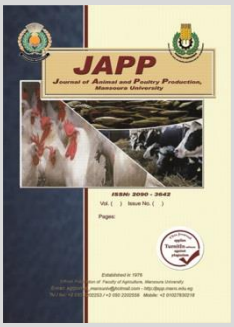

ABSTRACT

Current study aimed to examine possible acts of adding taurine or leptin to extender on frezability and fertiablity of frozen buffalo-semen. Five sexually mature buffalo male were used for semen collection (twice/week for 35 days) using artificial vagina. Mass motility of all collected ejaculates was ≥70%. The collected semen was pooled, allotted into 7 replicates, then extended in Tris-egg yolk without (control), with taurine and leptin (20 and 40 ng/ml for each), or two combination of taurine (10 and 20 ng/ml) and leptin (20 and 20 ng/ml). Semen was frozen in straws for 2 wk and thawed at 37^0C/30 seconds. In diluted, equilibrated and thawed semen, the live, individual motility and abnormal sperm percentages were determined, while the hypo-osmotic swelling test (curled tail) was examine in semen after thawing. In the thawed seminal plasma, enzyme activity was assayed. Fertilizing ability of each semen treatment was determined. Results showed that adding 20 ng leptin/ml or 40 ng taurine/ml enhanced (P<0.05) all spermatozoa parameters after dilution, equilibration and thawing versus control and other treatments. Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were reduced (P<0.05) with all additives compared with control semen. Pregnancy rate for buffalo cows which inseminated with extended semen contain 20 ng leptin /ml, 40 ng taurine /ml, and control was 90, 80 and 70% (P<0.05). Thia study concluded that, addition of 20 ng leptin/ml or 40 ng taurine/ml in the extender of Tris for buffalo frozen-semen had positive impact on sperm function during cryopreservation and increased fertility of buffalo-bull spermatozoa.

Keywords: Buffaloes, taurine, leptin, semen, cryopreservation, seminal plasma enzymes.

INTRODUCTION

Artificial insemination (AI), is an important tool in breeding of livestock. Good management during the collection and preservation is a limitation of AI program successful (Leboeuf *et al.*, 2000). Semen freezing is a way to slow the cellular metabolic activity, on the other hand it restart activity again after thawing (Medeiros *et al.*, 2002; Mazur, 1984).

In mammalian sperm, reduced the antioxidant defense system and sperm cells are depending on the seminal plasma antioxidant content (Martin-hidalgo *et al.*, 2019). In the seminal plasma of the semen, different antioxidants enzyme such catalase and super oxide dismutase and antioxidants not enzyme like glutathione, pyruvate, ascorbic acid, α-tocopherol, taurine and hypo-taurine are present in the antioxidant system. The protective effects of these antioxidants to protect sperm cells against the oxidative stress prior to cryopreservation significantly reduced diluted and equilibrated semen (Jong *et al.*, 2012).

Several sperm characteristics including motility, live and membrane integrity of sperm cells reduced in frozen semen, where reactive oxygen species (ROS). The male fertility was affected by Antioxidants which have a critical function in fertility. The Cold shock which happen throw the process of freezing or thawing might be cause damage for the mitochondria (Pena *et al.*, 2009), also plasma and acrosome membranes of spermatozoa (Meyers, 2005). Dead spermatozoa may be generating ROS in bovine semen via aromatic amino acid oxidase catalyzed reactions (Upreti, *et al.*, 1998). Oxidative stress occurs because of unbalance between antioxidant fortification and production of free radicals so the results were damage of tissue. Therefore adding exogenous antioxidant to semen diluent provided protective effect over mammalian and human sperm during preservation (Aurich *et al.*, 1997; Foote *et al.*, 2002). Adding antioxidant like taurine to semen extenders create a protection defense against ROS, so improved spermatozoa motility was explained by Bucak and Tekin (2007).

Mammalian sperm cells have sensitivity to the oxidative stress as affected by increasing level of O_2 which decrease the level of endogenous antioxidants. (Zhang *et al.*, 2015). Sperm membranes composed of high concentrations of polyunsaturated fatty acids like arachidonic and decosahexaenoic acids , it lead to sperm susceptibility to ROS, causing lipid peroxidation, which reduce sperm characteristics with more extend in buffaloes (Lenzi *et al.*, 2002; Nair *et al.*, 2006). Therefore, addition of antioxidants during semen dilution may enhance sperm quality during storage. The oxidative stress impaired effect can be decreased by antioxidant molecules consequently enhancing the quality of spermatozoa which follwingt the freezing or thawing process. The production of ROS can be reduced and prevent oxidative stress if we adding various antioxidants in semen extenders (Foote *et al.*, 2002; Funahashi and Sano, 2005).

Mitochondria defending against superoxide generation that can done by using taurine as antioxidant; to regulate mitochondrial protein synthesis (Jong *et al.*, 2017).The end

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product for cysteine metabolism in mammalian is turine also, it consider on of the most abundant of the low-molecular-weight organic constituents (Ekremoğlu *et al.*, 2007). Taurine and cysteine to be vine sperm (Bilodeau *et al.*, 2001 and sariozkan *et al.*, 2009), also play a big role to improve protection for spermatozoa against the harmful effect of ROS and improving post-thaw sperm motility, viability and fertility. Protection of the sperm cell against ROS it can be done by traverse the sperm plasma membrane, inhibit lipid peroxidation, (Tsuzuki *et al.*, 2010; Beheshti *et al.*, 2013). Several studies used Taurine as an antioxidant and evaluated it has positive improve on motility, viability and plasma membrane integrity for spermatozoa of different kind of animals (Uysal *et al.*, 2007; Foote *et al.*, 2002). During sperm cryopreservation or storage, taurine adding to semen of different species shown to improve sperm motility and membrane integrity in fresh state (Jang *et al.*, 2006; Bucak and Tekin, 2007Sariozkan *et al.*, 2009).

The white adipose tissue produces leptin which is consists from 167- amino acid , also his weight 16 KD a protein adipokine, a pleiotropic cytokine-like hormone. It is produced to regulate energy, homeostasis, oxidation of fatty acids, blood vessel, sexual maturity and propagation (Branian and Hansen 2002; du plessis *et al.*, 2010). Sperm cells have the ability for modulation their metabolism by secretion of leptin, based on energy requirements, affecting development of sperm motility (Aquila *et al.*, 2005; Lang Consiglio *et al.*, 2009). Therefore, leptin improve sperm parameter positively (Jorsaraei *et al.*, 2008 and 2010), and leptin supplementation to semen extender can maintain sperm characteristics in of buffalo semen preserved at cool temperature (Khaki *et al.*, 2013), or cryopreserved buffalo semen (Abdel-Khalek *et al.*, 2016). The effect of the combination between leptin and taurine on cryopreserved buffalo semen was not studied.

Therefore, the current study aimed to determine fertilizing or freezing ability of buffalo semen which may be affected by leptin or taurine or their combinations, as antioxidant additives, in Tris-based extender.

MATERIALS AND METHODS

This experiment was conducted in cooperation with Animal Production Research Institute, Egypt, and Department of Animal Production, Faculty of Agriculture, Kafer El-Sheikh University.

Animals:

Total of 5 Egyptian mature buffalo males (aged from 4 to 5 y and live weight for body ranged from 450 to 500 kg) all male were used to collect semen from them, in curent research. All bulls were sexually adult and free of reproductive diseases. They were individually fed on daily ration (8 kg concentrate feed mixture, and 12 kg rough included Berseem hay and rice straw). Bulls were fed twice/day at 8 a.m. And 4 p.m., the water was available ad lip the male housed separated one box for each bull.

Semen collection:

Twice time every weak we collect semen ejaculates from male by using artificial vagina for 5 weeks period, (IMV, France) maintained at appropriate temperature, before feeding between at 7-8 a.m. One male we used like a female to be mounted by other bull on collection day. Only semen ejaculates with mass motility more than 70% were directly taken in put immediately in bath which contain worm water at 37 ^{0}C inside the laboratory for evaluation and starting freezing processes. We pooled all collected ejaculates (n=5) consequently partition for 7 part for each treatments control, 2 concentrate for leptin, (20 and 40 ng/ml), 2 levels of taurine, (20 and 40 ng/ml) and a combination of taurine and leptin 20 and 40 ng/ml, respectively) (Sigma Aldrich Comp., St. Louis, Mo., USA).

Preparation of semen extenders and dilution:

The base extender of semen dilution was Tris- egg yolk, which composed of 0.325 g Tris, compose from 1.675g of citric acid, 0.75 g of glucose.We use 0.005g of streptomycin and 0.025 g of lincomycin as source of antibiotic. All content dissolved in 100 ml distilled water. To each 83 ml of Tris extender, 10% egg yolk also we add 7% glycerol as cryoprotectant were mixed. The Tris-base extender was divided into seven types with different supplements: unsupplemented (T1), taurine at levels of 20 ng/ml (T2), or 40 ng/ml (T3), leptin at levels of 20 ng/ml (T4) or 40 ng/ml (T5) and, combination of taurine and leptin at a ratio of 10 : 10 ng/ml (T6) or 20:20 (T7). Semen was diluted at a rate of 1:10.

Semen freezing:

After diluted the semen with different types of extender and additives (Turine and liptine), the diluted semen was aspirated into 0.25 ml French straws, sealed with polyvinyl alcohol power. Semen straws equilibration were done at 5^{0}C for 4 h, then frozen at 5 cm above surface with nitrogen vapour (for 10 min), then inserted and preservation inside tank full with liquid nitrogen (-196^{0}C) for 4 weeks.

Semen assessment:

Semen with each type of extender supplement was evaluated in diluted, equilibrated and frozen/thawed semen at 37^{0}C for 30 seconds in a water bath. Sperm parameters, including percentage of motility, live and abnormality of sperm cells were determined. A warm stage (at 37 ^{o}C) microscope was used for determination percentage of the motility of each sperm (Amman and Hammerstedt, 1980). Eosin and nigrosin mixture stain was used foe recording live sperm percentage (Hackett and Macpherson, 1965), while the sperm abnormality percentage was asses according to the classification adopted by Blom (1983). Post-thawing recovery rate of sperm motility was computed as the following:

The percentage of sperm motility recovered rate = (percentage of motility after thawing /initial motility percentage) x100.

Hypoosmotic swelling test (HOST):

After semen thawing, the HOS-t was performed using solution with the level of osmolarity of 150 mOsm/l for 30 min for determination sperm membrane integrity by employing the technique developed by Maxwell and Johnson, 1997)

The activity of enzyme in thawed seminal plasma:

Samples for semen after thawing were centrifuged at 4000 rpm for 15 minutes for separation of the seminal plasma, which was stored until analysis at -20 oC. Activities of asprtate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH) were assayed using spectrophotometer (JENWAY-6405UV/Vis) and commercial kits (Salucea Netherlands) and according to Young (1990)

Fertility trial:

A double dose (3 ml Estrumate i.m.) within 11 days, and AI throughout 48-72 h, (Estrumate PGF2α-Essex Animal Health Friesoythe, Germany) was conducted for induction estrus for 35 buffalo cows. Thirty buffalo cows which come in

heat were inseminated by frozen semen with leptin (20 ng/ml) or taurine (40 ng/ml) versus control semen according to the best obtained results. Immediately, after semen thawing (37 ºC/30 seconds), buffalo cows were artificially inseminated by using AI gun to deposit semen inside uterus throw cervix, so all insemination were carried out by the same inseminator. After 50 to 60 days post-insemination the non-return buffalo cows were diagnosis for pregnancy manually by rectal palpation.

Statistical analysis:

All data of sperm parameters and enzyme activity were analyzed by one way ANOVA using SPSS program version 2013 statistical analyses. We use Duncan multiple range teat (Duncan, 1955) to determine the differences are significant or not. The percentage values were subjected to arcsine transformation and treatment means were tabulated after being recalculated from the transformed values to percentages. Conception rate was analyzed by Chi-square test.

RESULTS AND DISCUSSION

Sperm parameters after semen dilution:

Table (1) shows that percentages of motility for each sperm in semen post-dilution significantly (P<0.05) increased in all treatments, except T2, versus control (T1), being the highest in T3 and T4. However, live and abnormal sperm percentages were significantly (P<0.05) improved by all antioxidant additives. Current results indicated that the use of 20 ng/ml of leptin or 40 ng/ml of taurine in Tris-extender showed beneficial effect on sperm parameters of diluted semen.

Table 1. Sperm parameters in buffalo semen as affected by taurine and leptin supplementation to Tris-extender after dilution.

Sperm Characteristics	Control (T1)	Taurine		Leptin		Taurine + Leptin	
		T2	T3	T4	T5	T6	T7
Sperm individual motility (%)	$70.12^{c}\pm0.51$	$72.75^{cb}\pm1.06$	$78.00^{a}\pm0.70$	$78.62^{a}\pm1.46$	$73.37^{cb}\pm0.73$	$74.87^{ab}\pm1.20$	$76.75^{ab}\pm2.83$
Live sperm (%)	$65.12^{c}\pm1.44$	$69.75^{b}\pm1.62$	$76.75^{a}\pm0.97$	$79.62^{a}\pm1.26$	$71.25^{b}\pm0.99$	$72.25^{b}\pm1.06$	$72.25^{b}\pm0.59$
Sperm abnormality (%)	$28.75^{a}\pm1.54$	$23.12^{b}\pm1.00$	$16.87^{de}\pm0.71$	$14.37^{e}\pm0.77$	$21.25^{bc}\pm0.83$	$19.50^{cd}\pm1.01$	$20.00^{c}\pm0.98$

[a-c]: Significant differences at P<0.05) for different superscripts in the same row.

Sperm parameters after equilibration:

As proved in Table (1) in semen after dilution, all parameters of sperm studied were improved significantly (P<0.05) in semen after equilibration, being the highest in T4. These results reflected the beneficial effects of semen dilution with Tris-extender supplemented liptine with level of 20 ng/ml on improving sperm functions in equilibrated semen.

Table 2. Sperm parameters in buffalo semen as affected by taurine and leptin supplementation to Tris-extender after equilibration.

Sperm characteristics	Control (T1)	Taurine		Leptin		Taurine + Leptin	
		T2	T3	T4	T5	T6	T7
Sperm individual motility (%)	$63.00^{c}\pm0.84$	$66.87^{b}\pm1.31$	$72.75^{a}\pm1.08$	$74.00^{a}\pm1.10$	$67.25^{b}\pm0.86$	$68.37^{b}\pm0.53$	$67.62^{b}\pm0.73$
Live sperm (%)	$59.75^{d}\pm1.95$	$63.87^{c}\pm1.63$	$72.12^{b}\pm1.10$	$77.12^{a}\pm0.78$	$65.25^{c}\pm1.26$	$66.62^{c}\pm1.10$	$65.25^{c}\pm1.29$
Sperm abnormality (%)	$35.00^{a}\pm1.16$	$29.75^{b}\pm1.19$	$20.87^{d}\pm0.66$	$17.50^{e}\pm0.62$	$29.25^{b}\pm1.26$	$23.87^{c}\pm0.71$	$26.37^{c}\pm0.94$

[a-c]: Significant differences at P<0.05) for different superscripts in the same row.

Sperm parameters after freezing/thawing:

Table (3) shows that all characteristics of spermatozoa in semen after freezing/thawing were significantly (P<0.05) improved by all antioxidant additions, better results found in T4. However, recovery rate of sperm motility after thawing significantly (P<0.05) increased only in T3 and T4 versus control (T1) and other additions.

The present results showed that motility, recovery rate of sperm motility, live sperm, abnormal sperm and sperm membrane integrity were improved significantly (P<0.05) after thawing in semen diluted with 20 ng leptin/ml.

The activity of enzymes inside seminal plasma after semen thawing:

Table (4) shows significant (P<0.05) reduction in AST, ALT and LDH activities in the thawed seminal plasma of semen supplemented with all types of antioxidants in comparing with free semen, being the least in T3.

Table 3. Sperm parameters in buffalo semen which affected by taurine and leptin adding to the Tris-extender after thawing.

Sperm parameter	Control (T1)	Taurine (ng/ml)		Leptin (ng/ml)		Taurine + Leptin	
		T2	T3	T4	T5	T6	T7
Sperm individual motility (%)	$42.87^{d}\pm1.43$	$47.62^{c}\pm1.43$	$55.62^{b}\pm1.84$	$60.50^{a}\pm0.86$	$48.12^{c}\pm1.12$	$49.62^{c}\pm1.16$	$48.50^{c}\pm1.16$
Recovery rate of sperm motility	$61.06^{c}\pm1.62$	$65.59^{bc}\pm2.32$	$71.21^{b}\pm1.77$	$77.01^{a}\pm0.68$	$65.58^{bc}\pm1.41$	$66.40^{bc}\pm1.92$	$63.85^{c}\pm2.94$
Live sperm (%)	$45.00^{d}\pm1.41$	$46.37^{c}\pm1.49$	$52.62^{b}\pm1.16$	$62.50^{a}\pm0.80$	$46.62^{c}\pm1.43$	$49.12^{bc}\pm1.02$	$47.25^{c}\pm1.34$
Sperm abnormality (%)	$39.00^{a}\pm1.22$	$35.50^{b}\pm1.34$	$21.37^{c}\pm0.82$	$18.00^{d}\pm1.00$	$35.25^{b}\pm1.34$	$33.50^{b}\pm0.86$	$34.12^{b}\pm0.95$
Membrane integrity (%)	$39.12^{c}\pm1.00$	$45.62^{b}\pm1.14$	$48.12^{ab}\pm0.91$	$50.37^{a}\pm1.26$	$46.50^{b}\pm1.06$	$47.00^{b}\pm1.11$	$46.75^{b}\pm0.97$

[a-c]: Significant differences at P<0.05) for different superscripts in the same row.

Table 4. The activity of enzymes inside seminal plasma after buffalo semen thawing semen ass affected by taurine and leptin supplementation to Tris-extende.

Activity	Control (T1)	Taurine (ng/ml)		Leptin (ng/ml)		Taurine + Leptin (ng/ml)	
		T2	T3	T4	T5	T6	T7
AST (U/L)	$88.75^{a}\pm1.49$	$59.25^{b}\pm0.49$	$52.25^{cd}\pm1.60$	$49.50^{d}\pm0.64$	$58.50^{b}\pm0.64$	$54.50^{c}\pm1.70$	$54.75^{c}\pm1.03$
ALT (U/L)	$52.25^{a}\pm1.31$	$41.50^{b}\pm0.28$	$37.25^{c}\pm1.10$	$33.25^{d}\pm1.18$	$40.75^{b}\pm0.25$	$39.75^{bc}\pm0.47$	$40.50^{b}\pm0.64$
LDH (U/L)	$377.50^{a}\pm3.12$	$341.75^{b}\pm1.37$	$318.50^{c}\pm3.37$	$305.75^{d}\pm4.04$	$340.50^{b}\pm1.55$	$335.50^{b}\pm2.10$	$337.00^{b}\pm2.34$

[a-d]: Significant differences at P<0.05) for different superscripts in the same row.

Fertility trial:

Data in Table (5) cleared that conception rate (CR) for buffaloes cow following artificial insemination with semen diluted by Tris-extender which contain leptin at a level of 20 ng/ml was higher (90.0%) significantly (P<0.05) versus control (70%). Also, CR of buffalo cows inseminated by semen with taurine at a level of 40 ng/ml (80.0%) was significantly (P<0.05) higher than the control. The difference between both treatment groups was significant (Table 5).

Table 5. Effect of taurine and leptin supplementation to Tris-extender on conception rate of buffalo cows.

Item	Post-thawed semen		
	Control	Taurine (40 ng/ml)	Leptin (20 ng/ml)
Inseminated animals, n	10	10	10
Conceived animals, n	7	8	9
Conception rate	70.0^{c}	80.0^{b}	90.0^{a}

$^{a-c}$: Significant differences at P<0.05) for different superscripts in the same row.

Discussion

There is a deficiency in cytoplasmic component which have antioxidant effects to expunge ROS, therefore, the capability in sperm cells antioxidant is limiting. Thus, mammalian sperm is not capable enough to counteract peroxidation during the freezing and process of thawing (Bilodeau *et al.*, 2000; Lapointe and Bilodeau, 2003; Alvarez and Storey, 2005). Therefore, the sperm plasma membranes are vulnerable to ROS damage due to their high polyunsaturated fatty acid content and the use of antioxidants could reduce the negative ROS impact on spermatozoa.

Taurine is already found in most tissues of mammalian, having an impotent and valuable role in viability, cell proliferation, osmo-regulation, and prevention of possible injuries which is cause by oxidants in many tissue(Chesney, 1985).

The present study aimed to evaluate the effect of leptin and turine added at two levels into the buffalo-semen extender on sperm characteristics in diluted, equilibrated and frozen/thawed buffalo-semen. Results revealed that addition of leptin (20 ng/ml) and turine (40 ng/ml) to Tris-base extender for the semen of buffalo-during cryopreservation has beneficial effects on sperm function directly. These results like those obtained by Khaki *et al.* (2013), who found that adding 10 ng leptin/ml in extender of cryopreserved buffalo-semen significantly maintained sperm motility parameters, and viability in equilibrated semen as compared to control or other leptin levels. However, insignificant effect of leptin addition was observed on sperm DNA degeneration and sperm membrane integrity. In the present study, increasing level of leptin to 20 ng/ml leads to additional benefits on sperm function directly, in term of increasing membrane integrity in post-thawed semen. The semen parameters were improved by adding antioxidant like turine and trehalose to different animal semen extenders has been reported. Turine, plays as non-enzymatic antioxidant for protecting sperm cells against ROS. The current study showed that adding taurine at a level of 40 ng/ml to buffalo semen extender was unlikely to act on sperm function directly. In accordance with the present results, El-sheshtawy *et al.* (2008) found that adding special amino acids like glutamine, glycine, alanine and systein to extenders before freezing causes improvement of semen quality after thawing.

Several investigators (Funahashi and Sano, 2005; Uysal *et al.*, 2007; Atessahin *et al.*, 2008; Bucak and Uysal, 2008) showed improved motility after freezing in different animal species as affected by taurine addition to semen extenders prior to freezing process. Camina *et al.* (2002), Glander *et al.* (2002), and Lackey *et al.* (2002) reported that the seminal plasma of human contains leptin and the accessory sex organs in males mainly secreted leptin. In human, Lampiao and du Plessis (2008) and Jorsaraei *et al.* (2008) found that total and progressive sperm motility as well as acrosome reaction and nitric oxide production increased by *in vitro* addition of leptin. Leptin treatment may stimulate the cholesterol efflux and activity of acrosin in sperm cells, which may explain the physiological role of leptin to improve sperm function parameters in this study.

Increasing fertility of semen with leptin may be attributed to the association between the leptin secretions and increased capacitated spermatozoa. In this respect, Abdel-Khalek *et al.* (2016) showed positive effects of adding leptin (20 ng/ml) to Tris- extender on sperm parameters of buffalo-semen after dilution, equilibration and thawing, reflecting marked increase in pregnancy rate in of buffalo cows. Also, Garcia-Mayor *et al.* (1997) and Bado *et al.* (1998) stated that leptin plays important roles in spermatogenesis and sperm capacitation, which indicates the beneficial effect of leptin on pregnancy rate of buffalo cows in the present study. It is worthy noting that improving sperm parameters by addition of leptin (20 ng/ml) or taurine (40 ng/ml) in semen extenders was associated with remarkable decrease in enzyme release such as AST, ALT and LDH. Generally, leptin plays an important role in improving the fertilizability of human sperm by improving motility and acrosome reaction of spermatozoa (Lampiao and du Plessis, 2008).

Based on the foregoing results, addition of leptin (20 ng/ml) or turine (40 ng/ml) to Tris-based extender increased freezability and fertilizability of buffalo spermatozoa. These supplementations can be helpful for artificial insemination in buffaloes.

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التأثير المضاد للأكسدة لإضافة كل من التورين واللبتين فى مخفف الترس على القدره التجميديه والإخصابية للسائل المنوى للجاموس

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أجريت هذه الدراسة بالتعاون بين كلية الزراعة بكفر الشيخ – قسم الانتاج الحيوانى ومعهد بحوث الإنتاج الحيوانى. وتهدف الدراسة الى اختبار التأثيرات الممكنه عند استخدام التورين واللبتين كمضادات أكسدة لمخفف الترس أثناء تجميد قذفات السائل المنوى لذكور الجاموس الناضجة . إستخدم في هذه الدراسة 5 ذكور جاموس متوسط اعمارها من 4 الى 5 سنوات ، وكان أوزانها ما بين (450 – 500) كجم، تم إستخدام المهبل الصناعى لتجميع قذفات السائل المنوي الذكور بعد 2مرة أسبوعيا و لمدة خمسة أسابيع بمعدل40 قذفة لكل الذكور المستخدمة فى الدراسة و كان متوسط النسبة المئوية للحركة الجماعية للحيامن من القذفات المجمعة حوالى 70% أو أكثر. تم خلط جميع القذفات لكل يوم جمع تم تقسيم القذفات المجمعه الي عدد سبع مكرارت للمعاملات المختلفة وتم تقسيمها كما يلى: المعاملة الاولى (مخفف الترس وحده) ، المعاملة الثانية(تم إضافة التورين بمعدل 20نانوجرام/مل) والمعاملة الثالثة (أضيف التورين بمعدل 40 نانو جرام / مل)، المعاملة الرابعة(تم اضافة اللبتين بمعدل 20 نانو جرام /مل) و المعاملة الخامسة (اضيف اللبتين بمعدل40 نانو جرام / مل) ، المعاملة السادسة (و السابعة (اضافة التورين مع اللبتين بمستويات 20 و 40 نانو جرام / مل) . و لقد كانت نسبة السائل المنوى إلى المخفف بنسبة 1: 10 تم عمل موازنة للسائل المنوى المخفف لمدة 4 ساعات على 5 درجة مئوية تلى التجميد فى النتروجين السائل تم حفظ الجرعات بالقصيبات فى النتروجين السائل لمدة 4 أسابيع تلى ذلك عمل اسالة لقصيبات السائل المنوي المجمده على درجة 37 مئوية لمدة 30 ثانية وذلك لكل المعاملات بغرض عمل تقييم للسائل المنوى بعد التخفيف وبعد عملية الموازنة وبعد الاسالة. ولتقدير النشاط الانزيمى فى بلازما السائل المنوى وتشمل انزيمات AST, ALT, LDH بعد كل من عملية التجميد والاسالة ، وتم عمل تجربة تلقيح باستخدام باستخدام مخففات (المعاملة الاولى- الثالثة – الرابعة) حيث تم تلقيح 10 أناث من الجاموس صناعيا وتم تقدير معدل الحمل. وأشارت النتائج إلي: 1- تحسنت جميع خصائص الحيوانات المنويه عند معنوية بمستوى (0.05 %) باضافة اللبتن بتركيز 20 نانوجرام/ مل أنخفض النشاط الانزيمي لكل من AST,ALT,LDH فى البلازما المنويه بعد الاسالة في كل المعاملات مقارنة بالكنترول وكانت أقل الأنخفاضات مع اللبتن بتركيز 20 نانوجرام / مل عند بمعنوية مستوي (0.05 %). اوضحت النتائج المتحصل عليها أن أعلى معدل للحمل كان عند استخدام اللبتن بمعدل 20 نانوجرام / مل يليه التورين بمعدل 40 نانوجرام / مل مقارنة بالكنترول بنسبة مئوية (90.0 ، 80.0 و 70.0 % علي التوالي). وأظهرت هذه الدراسة أن إضافة أى من اللبتين (20 نانوجرام / مل) والتورين (40 نانوجرام/مل) إلى مخففات الترس كمضادات أكسدة قد أدى إلى تحسين جودة وخصوبة السائل المنوى المجمد لذكور الجاموس.