Effect of methyl β cyclodextrin on freezability and in vitro fertilizing potential of buffalo spermatozoa

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Abstract

Cryopreservation induces partially irreversible damage to sperm membrane as a result of membrane destabilization. The membrane damage after freezing might be linked to the modification of the membrane fluidity before freezing. Methyl β cyclodextrin (mβCD) was found to selectively extract cholesterol from the plasma membrane and therefore, increased membrane fluidity. The present study aimed at investigating the effect of adding mβCD to the Tris-based diluent on the freezability and in vitro fertilizing potentials of buffalo spermatozoa. Buffalo semen was collected, evaluated and extended in Tris-based extender supplemented with different concentrations of mβCD (experiment 1) or with cholesterol-3-sulfate and/or mβCD (experiment 2). Semen was examined post-thawing to evaluate their freezability, cholesterol content and in vitro fertilizing potentials. The present results revealed that, addition of mβCD to the semen diluent before freezing improved significantly the freezability and fertilizing potentials in a dose dependent trend. Addition of 5 mg/ml mβCD increased significantly ($P<0.01$) post-thawing motility, viability index and maintained the acrosomal integrity (57.50±2.50%, 157.50±8.29 and 12.00±0.81%, respectively) compared to the control semen (41.25±1.25%, 105.55±2.88 and 18.25±2.46%, respectively) and increased significantly ($P<0.05$) the development to morula and blastocyst stage (24.44% and 15.56%, respectively) compared to the control semen (6.38 % and 2.13%, respectively). Moreover, 5 mg/ml mβCD could delete the drastic effect of cholesterol-3-sulfate on the buffalo semen freezability and in vitro fertilizing potentials.

In conclusion, addition of methyl β cyclodextrin to the semen diluent prior to freezing is an effective method that increases the cryosurvival and in vitro fertilizing potentials of buffalo spermatozoa.

INTRODUCTION

Cryopreservation induces partially irreversible damage to the sperm plasma membrane. Part of this damage occurs as a result of alteration of the lipid content and phospholipids release ***(Oliw et al., 1993 and Moore et al., 2005)***. These changes of plasma membrane lipid composition have been responsible for the change in the sperm membrane fluidity upon cryopreservation ***(Canvin and Buhr, 1989 and Buhr et al., 1994).*** Within the plasma membrane of sperm cell, cholesterol molecules impart stability to the lipid bilayers by maintaining a random arrangement of phospholipids and transmembrane proteins ***(McGrath, 1988 and Moore et al., 2005)***. Cholesterol-phospholipids ratio in the sperm membrane may infact play a mechanistic role in membrane stability and appears to influence the fertilizing potentials of the mammalian spermatozoa ***(Langlais and Roberts, 1985; Grippo et al., 1994; Hartel et al., 1998 and Badr et al., 2004)***. The removal of this sterol could account for the increase in the fluidity and permeability of biomembranes ***(Cooper et al., 1978),*** which would provide immediate protection against cryoinjury ***(Barrera-Compean et al., 2005).*** Methyl β cyclodextrin (mβCD), a cyclic oligosaccharide consisting of 7 β (1-4)-glucopyranose unit, was found to selectively extract cholesterol from the plasma membrane in preference to other membrane lipids ***(Iborra et al., 2000; Shadan et al., 2004 and Barrera-Compean et al., 2005)***. Methyl β cyclodextrin stimulates the efflux of membrane cholesterol in a dose – dependent manner, in mouse ***(Choi and Toyoda, 1998 and Visconti et al., 1999);*** goat ***(Iborra et al., 2000)*** and bull ***(Marie, 2005 and Purdy et al., 2005)*** spermatozoa. The fact that mβCD functions as a sink for cholesterol, raises the possibility that mβCD enhances the freezability of spermatozoa by increasing membrane fluidity ***(Moore et al., 2005 and Moće and Graham 2006).*** However, the relationship between membrane fluidity before freezing and cryosurvival of buffalo spermatozoa

are poorly understood. Therefore, the aim of the current study was to investigate the effect of mβCD on cryosurvival of buffalo spermatozoa and their subsequent in vitro fertilizing potentials.

Material and Methods

Semen collection and extender:

Semen samples were collected from six buffalo bulls of proven fertility, kept at the Animal Reproduction Research Institute farm, Al-Harm, Egypt. Only semen samples of at least 70 % initial motility and 800.00X10^6sperm cells/ml were used. Immediately after collection, semen samples were pooled, split into 9 portions and diluted at a 1:8 ratio at 30 ^{o}C with Tris-based extender ***(Abdel-Malak et al., 1994)***. In experiment 1, the extender was supplemented with, 0, 1, 3, 5, 10 or 15 mg/ml mβCD. In experiment 2, the extender was supplemented with, 0, 10 mg/ml cholesterol-3-sulfate and/or the best concentration of mβCD from experiment 1.

Processing of buffalo semen:

Immediately after dilution, the extended semen was cooled to 5 ^{o}C throughout 60 minute in a cold cabinet. The cooled semen was loaded into 0.25 ml French straws (IMV, L'Aigle, France), then lowered into liquid nitrogen vapor inside foam box according to ***Mohammed et al. (1998).*** The straws were then immersed into liquid nitrogen and stored.

Evaluation of semen freezability:

Frozen semen samples were thawed in a water bath at 40 ^{o}C for 30 second. Sperm motility was assessed subjectively, immediately after dilution, post-thawing and after 1, 2 and 3 hours of thawing. Post-thawing viability indices were recorded according to ***Milovanov (1962).*** Acrosomal defects were recorded after thawing in smears stained by Fast Green (FCF) according to ***Wells and Awa (1970).***

Estimation of cholesterol efflux:

Cholesterol efflux from spermatozoa was estimated by determination of cholesterol concentration in the diluent. Cholesterol concentration (mg/dl) was determined according to ***Allain et al. (1974).***

Evaluation of in vitro fertilizing potential of the treated semen:

Frozen semen treated with mβCD and/or cholesterol at various concentrations was used to evaluate the fertilizing potentials of the treated semen in vitro.

In vitro oocyte maturation:

Buffalo ovaries were collected from an abattoir and transported to the laboratory in a sterile physiological saline at approximately 30 ^{o}C within 4 h of slaughter**.** Cumulus-oocyte complexes (COCs) were collected by aspiration of buffalo ovarian follicles. Oocytes with homogenous ooplasm and surrounded by multi-layers of compact cumulus cells, were selected for in vitro maturation. The selected oocytes were cultured in TCM-199 medium (Earl's salt, Sigma Chemical CO., St. Louis, Mo., USA) supplemented with 10% fetal calf serum (FCS,Gibco, 30 K-0351), 10μg/ml Luteinizing hormone, 5 μg/ml follicle stimulating hormone and 1μg/ml 17β estradiol. The oocytes were cultured for 24 hour at 38.5 ^{o}C in an atmosphere of 5% CO_2 in air with maximum humidity as cited by ***Badr (2001).***

Sperm preparation and oocyte insemination in vitro:

Three straws from each treatment were thawed in a water bath at 40 ^{o}C for 30 sec. Immediately after thawing, the most motile spermatozoa were separated by swim up technique in sperm-TALP medium containing 6 mg/ml bovine serum albumin (BSA), for 1 hour ***(Parrish et al., 1988)***. The uppermost layer of the medium containing the most spermatozoa was collected. The selected spermatozoa were washed twice by centrifugation at 2000 rpm for 10 minutes. The sperm pellet was resuspended in the fertilization TALP medium containing 10 μg/ml heparin. After appropriate dilution, 2 μl of sperm suspension was added to the fertilization drops, containing matured oocytes, at a final concentration 2 X10^{6} sperm cell/ml. Gametes were co-incubated in the fertilization drops under sterile mineral oil for 18 hour at 38.5 ^{o}C in an atmosphere of 5% CO_2 in air with maximum humidity. At the

end of gametes co-incubation, some of inseminated oocytes were fixed in acetic acid-ethanol (1:3) and stained with 1% aceto-orcein stain and examined under phase-contrast microscope (X 400) for evaluating the in vitro fertilization rate according to ***Totey et al. (1993)***. The presence of the second polar body, swollen or decondensing sperm head or even a detached sperm tail in the ooplasm was regarded as an evidence of sperm penetration. Penetrated oocytes with a male pronucleus or male and female pronuclei were regarded as an evidence of fertilization.

In vitro culture:

The inseminated oocytes were freed from cumulus cells and the attached spermatozoa by gentle pipetting and cultured in TCM-199 medium with Hepes modification for 7- days at 38.5 ^{o}C in an atmosphere of 5% CO_2 in air with maximum humidity. The proportional of cleaved oocytes was recorded 48 hour after insemination and those developed to the morula and blastocyst stages were recorded at 5-7 day post-insemination according to ***Totey et al. (1992***).

Statistical analysis:

All data were analyzed by using ***Costat Computer Program, Version 3.03 copyright (1986) Cottort Software***, and were compared by the least significant difference least (LSD) at 1% and 5% levels of probability. In vitro fertilization rate and embryo development were analyzed by chi-square analysis (X^2).

Results

Data presented in table 1 revealed that, the addition of mβCD to the semen extender before freezing improved the freezability of buffalo semen compared to the control semen. This improvement appeared to be dose-dependent trend. Addition of 5 mg/ml mβCD to buffalo semen extender was the best concentration that enhanced sperm freezability and increased significantly ($P<0.01$) post-thawing motility, viability index and maintained the acrosomal integrity (57.50±2.50%, 157.50±8.29 and 12.00±0.81%, respectively) compared to the control semen (41.25±1.25%, 105.00±2.88 and 18.25±2.46%, respectively). Moreover, the current results demonstrated that, increasing concentration of mβCD, maintained higher percentages of motile and viable sperm up to 15 mg/ml when the percentages of motile, viable sperm

and the acrosomal integrity began to decline sharply (32.50±4.87, 67.50±10.30 and 19.00±3.13%, respectively).

Table 1: Effect of adding methyl β- cyclodextrin to semen extender on sperm characters of frozen buffalo semen (Mean±SE).

Different treatments	Immediate dilution motility (%)	motility after1 hour dilution (%)	Post-thawing motility (%)	Viability index	Acrosomal abnormality (%)
Control	82.50±2.5 **ab**	82.50±1.44 **a**	41.25±1.25 **bc**	105.00±2.88 **b**	18.25±2.46 **ab**
mβCD 1 mg/ml	83.75±1.25 **ab**	83.75±1.25 **ab**	50.00±4.08 **ab**	122.50±16.89**ab**	15.50±0.95**abc**
mβCD 3 mg/ml	86.25±2.39 **ab**	87.50±1.44**ab**	52.50±4.78 **ab**	146.25±16.75 **a**	13.00±1.22 **bc**
mβCD 5 mg/ml	87.50±1.44 **a**	88.75±1.25 **a**	57.50±2.50 **a**	157.50±8.29 **a**	12.00±0.81 **c**
mβCD 10 mg/ml	86.25±1.25 **ab**	86.25±2.39 **ab**	52.50±4.78 **ab**	146.25±11.43 **a**	18.25±0.62 **ab**
mβCD 15 mg/ml	81.25±1.25 **b**	83.75±1.25 **ab**	32.50±4.87 **c**	67.50±10.30 **c**	19.00±3.13 **a**
Overall means	85.00±0.80	86.00±0.77	49.00±2.60	128.00±9.08	15.55±0.90

mβCD: methyl β cyclodextrin.
Values with different letters in the same columns are significantly different at least (P<0.05).

Experiment 2:

Data presented in table 2 showed that, addition of 10 mg/ml cholesterol-3-sulfate to the semen extender before freezing resulted in a significant (P<0.001) reduction in the post-thawing motility and viability index (27.50±4.78% and 42.50±10.89, respectively) compared to the control semen (41.25±1.25% and 105.00±2.88, respectively). However, addition of 5 mg/ml mβCD to the semen extender containing cholesterol-3-sulfate, deleted the drastic effect of cholesterol-3-sulfate, dependining upon cholesterol concentration in the semen diluent. Addition of 5 mg/ml mβCD to the semen diluent containing 1mg/ml cholesterol-3-sulfate resulted in a significant increase in the post-thawing motility and viability index (50.55±4.08% and 116.25±9.43, respectively) compared to addition of cholesterol-3- sulfate alone to the semen diluent (27.50±4.78% and 42.50±10.89, respectively). Whereas, addition of 5

mg/ml mβCD to the semen diluent containing 10 mg/ml cholesterol-3-sulfate did not improve buffalo semen freezability.

Table 2: Effect of adding methyl β-cyclodextrin to semen extender in the presence of low or high cholesterol-3-concentrations on sperm characters of frozen buffalo semen (Mean±SE).

Different Treatments	Immediate dilution motility (%)	motility after 1 hour dilution (%)	Post-thawing motility (%)	Viability index	Acrosomal abnormality (%)
Control	82.50±2.50 **a**	82.50±1.44 **a**	41.25±1.25 **ab**	105.00±2.88 **a**	18.25±2.46 **a**
Cholesterol-3-sulfate 10mg/ml	86.25±2.39 **a**	83.75±1.25 **a**	27.50±4.78 **c**	42.50±10.89	27.50±4.59 **a**
mβCD 5mg/ml+ Cholesterol-3-sulfate 1mg/ml	83.75±1.25 **a**	82.50±1.44 **a**	50.00±4.08 **a**	116.25±9.43 **a**	17.75±2.75 **a**
mβCD 5mg/ml+ Cholesterol-3-sulfate 10 mg/ml	86.25±1.25 **a**	82.50±1.44 **a**	40.00±7.07 **b**	66.25±8.50 **b**	23.25±3.25 **a**
Over all mean	85.41±0.96	82.91±0.74	39.16±3.97	75.00±10.55	22.83±2.23

mβCD: methyl β cyclodextrin.
Values with different letters in the same columns are significantly different at least (P<0.05).

Effect of adding methyl β- cyclodextrin to semen extender on cholesterol efflux:

The results presented in table 3 showed that after dilution for 1 hour and post-thawing, cholesterol concentration increased significantly in the diluent with the addition of 5 mg/ml mβCD (410.29±18.84 and 724.26±84.46 mg/dl, respectively) compared to the control semen or all other treatments except 10 mg/ml mβCD. These results revealed that cholesterol efflux from spermatozoa to the diluent was enhanced with the addition of 5 mg/ml mβCD. Moreover, the lowest cholesterol efflux was recorded with the addition of 10 mg/ml cholesterol-3- sulfate to the semen diluent either after cooling or post-thawing (182.05±13.32 and 349.47±15.62 mg/dl, respectively). However, addition of 5 mg/ml mβCD to the semen diluent containing 1mg/ml cholesterol-3-sulfate lead to improvement in the cholesterol efflux from the spermatozoa.

Table 3: Effect of adding methyl β-cyclodextrin to semen extender in the presence of low or high cholesterol-3- sulfate concentrations on total cholesterol efflux (Mean±SE).

Different treatment	Total cholesterol efflux (mg/dl)		
	Immediately after diluation	After1 hour dilution	Post-thawing
Control	290.75±2.06 **a**	323.55±2.67 **b**	389.32±13.83 **bc**
MβCD 1 mg/ml	124.03±5.64 **d**	246.25±20.02 **c**	389.32±13.80 **bc**
MβCD 3 mg/ml	150.60±15.16 **cd**	291.82±24.42 **bc**	473.35±44.24 **bc**
MβCD 5 mg/ml	189.43±3.06 **bc**	410.29±18.84 **a**	724.26±84.46 **a**
MβCD 10 mg/ml	157.31±13.83 **cd**	351.39±14.53 **ab**	525.76±51.86 **ab**
MβCD 15 mg/ml	229.74±12.35 **b**	330.18±2.60 **b**	486.39±20.74 **bc**
Cholesterol-3-sulfate 10mg/ml	225.80±0.34 **b**	182.05±13.32 **d**	349.47±15.62 **c**
MβCD 5mg/ml+ Cholesterol-3-sulfate 1mg/ml	212.92±5.13 **b**	325.75±12.80 **b**	386.86±1.21 **bc**
MβCD 5mg/ml+ Cholesterol-3-sulfate 10 mg/ml	152.54±11.56 **cd**	288.26±11.24 **bc**	353.06±11.37 **bc**

mβCD: methyl β cyclodextrin.

Values with different letters in the same columns are significantly different at least (P<0.05).

Data presented in table 4 showed that, addition of mβCD to the semen diluent improved significantly the penetration and the fertilization rates in a dose dependent trend. Addition of 5 mg/ml mβCD to the semen diluent significantly increased the fertilization rate (51.79%) compared to the control (29.31%), 10 mg/ml cholesterol-3-sulfate (28.33%) and 5 mg/ml mβCD + 10 mg/ml cholesterol-3-sulfate (38.09%). Addition of 10 mg/ml cholesterol-3-sulfate resulted in a significant decrease (P<0.05) in the penetration and fertilization rates (41.67 and 28.33 %, respectively). However, addition of combination of 5 mg/ml mβCD + 1 mg/ml cholesterol-3- sulfate to the

semen diluent improved the penetration and the fertilization rates nearly similar to the control semen.

Table 4: Effect of adding methyl β- cyclodextrin to semen extender on the in vitro penetration and fertilization rates.

Different treatments	No.of matured oocytes	Penetration rate (%)	Fertilization rate (%)
Control	58	31 (53.45) **a**	17 (29.31) **ab**
mβCD 1 mg/ml	65	40 (61.34) **a**	28 (43.08) **ab**
mβCD 3 mg/ml	65	43 (66.15) **a**	27 (41.54) **ab**
mβCD 5 mg/ml	56	36 (64.29) **a**	29 (51.79) **a**
mβCD 10 mg/ml	65	42 (64.62) **a**	31 (47.69) **a**
mβCD 15 mg/ml	60	38 (63.33) **a**	23 (38.33) **ab**
Cholesterol-3-sulfate 10mg/ml	60	25 (41.67) **b**	17 (28.33) **b**
mβCD 5mg/ml+ Cholesterol-3-sulfate 1mg/ml	63	35 (55.56) **ab**	24 (38.09) **ab**
mβCD 5mg/ml+ Cholesterol-3-sulfate 10 mg/ml	56	29 (51.79) **ab**	17 (38.09) **ab**

mβCD: methyl β cyclodextrin.

Values with different letters in the same columns are significantly different at least ($P<0.05$).

The results presented in table 5 demonstrated that, addition of mβCD to the semen diluent prior to freezing significantly increased the cleavage rate, the morula and the blastocyst development, in a dose dependent manner. Addition of 5 mg/ml mβCD to the semen diluent significantly increased ($P< 0.05$) the cleavage rate, the morula and the blastocyst development (48.89, 24.44 and 15.56%, respectively) compared to the control, 15 mg/ml mβCD and 10 mg/ml cholesterol-3-sulfate. On the other hand, addition of 10 mg/ml cholesterol-3-sulfate to the semen diluent resulted in a detrimental effect on embryo development in vitro. However, addition of combination of 5 mg/ml mβCD + 1mg/ml cholesterol-3-sulfate significantly

improved the morula development compared to the control and 10 mg/ml cholesterol-3- sulfate (13.33% vs 6.38 and 7.50%, respectively).

Table 5: Effect of adding methyl β- cyclodextrin to semen extender on the in vitro development of buffalo embryos.

Different treatments	No.of inseminated oocytes	Cleavage rate (%)	Morula (%)	Blastocyst (%)
Control	47	11 (23.40) **b**	3 (6.38) **b**	1 (2.13) **b**
mβCD 1 mg/ml	40	14 (35.00) **ab**	6 (15.00) **a**	3 (7.50) **a**
mβCD 3 mg/ml	45	17 (37.78) **ab**	8 (17.78) **a**	4 (8.89) **a**
mβCD 5 mg/ml	45	22 (48.89) **a**	11 (24.44) **a**	7 (15.56) **a**
mβCD 10 mg/ml	47	20 (42.55) **ac**	10 (21.28) **a**	5 10.64) **ab**
mβCD 15 mg/ml	42	15 (35.71) **ab**	4 (9.52) **a**	1 (2.38) **b**
Cholesterol-3-sulfate 10mg/ml	48	11 (22.92) **b**	2 (4.17) **b**	1 (2.08) **b**
mβCD 5mg/ml+ Cholesterol-3-sulfate 1mg/ml	45	14 (31.11) **ab**	6 (13.33) **a**	3 (6.67) **ab**
mβCD 5mg/ml+ Cholesterol-3-sulfate 10 mg/ml	42	11 (26.19) **bc**	3 (7.50) **b**	2 (4.76) **ab**

mβCD: methyl β cyclodextrin.
Dissimilar letters within the same column indicate significantly different values at P<0.05

Discussion

Cryopreservation induces partially irreversible damage to sperm plasma membrane as a result of reorganization of the membrane lipids and proteins. This membrane alteration, induced by the membrane changing from the fluid crystalline phase to the gel phase, as temperature is reduced lower than the membrane transition temperature, resulting in a rigid plasma membrane that apt to survive freezing ***(Moore et. al., 2005).*** One of the most efficient methods to prevent this membrane damage is to increase the membrane fluidity at low temperature. The lipid treatment prior to freezing makes the plasma membrane more flexible at lower temperatures and the sperm respond to the physical stresses better ***(Barrera-Compean et. al., 2005).*** These observations may explain the results of the current study, that emphasize that, addition of methyl β-

cyclodextrin to the diluent prior to cryopreservation significantly improved the freezability and the fertilizing potential of the buffalo-bull spermatozoa in a dose dependent trend. These results are in consistent with ***Zeng and Terada (2000); Moore et al. (2005); Galantino – Homer et al. (2006) and Li et al. (2006).*** The beneficial effect of methyl β cyclodextrin on the sperm function may be attributed to its ability to act as a sink for the cholesterol from the sperm plasma membrane ***(Kilsdonk et al., 1995; Christian et al., 1997; Barrera-Compean et al., 2005 and Marie, 2005)***. Membrane cholesterol efflux induces an enhanced membrane fluidity and permeability ***(Grunze and Deuticke, 1974; Cooper et al., 1978 and Purdy et al., 2005).*** By increasing membrane fluidity and permeability the amount of intracellular ice formation, which is lethal to the cell in cryopreservation, will be decreased ***(Mazur, 1985 and Essmail et al., 2004).*** Furthermore, the removal of sperm plasma membrane cholesterol content could be useful to protect sperm during cryopreservation by improving viability without promoting premature capacitation ***(Galantino – Homer et al., 2006)***. Nevertheless, the parallel correlation between the improvement of sperm cryosurvival and the increased membrane fluidity appears to be limited. The present results show that, high concentration of methyl β cyclodextrin resulted in a detrimental effect on sperm function. These results are in accordance with ***Purdy et al. (2005)***, who found that, cell possessing the highest membrane fluidity did not survive cooling and cryopreservation efficiently.

On the other hand, the present study reveals that, addition of cholesterol-3-sulfate to the semen diluent has a drastic effect on buffalo semen freezability and in vitro fertilizing potentials. These results are in agreement with ***Grippo et al. (1994)*** and ***Badr et al. (2004)***. This may be attributed to; the elevation of membrane cholesterol is associated with decreased membrane fluidity and subsequently increased the susceptibility of spermatozoa to cryoinjury ***(Madden and Quinn, 1979; Hartel et al., 1998 and Essmail et al., 2004).***

However, the addition of 10 mg/ml methyl β cyclodextrin to the semen diluent that contained 1 mg/ml cholesterol-3-sulfate improved buffalo semen function. These results are in consistent with ***Choi and Toyoda (1998); Iborra et al. (2000) and Zeng and Terada (2000),*** who reported that, methyl β cyclodextrin can stimulate the removal

of membrane cholesterol in mouse, goat and boar spermatozoa, when the concentration of extra cellular cholesterol is low.

Furthermore, the present results demonstrate that, the addition of methyl β cyclodextrin to the semen diluent resulted in a significant increase in sperm-oocytes penetration, fertilization and further buffalo embryo development in vitro. These results are in agreement with ***Purdy and Graham (2004)*** and ***Moore et al. (2005)***, who reported that, addition of methyl β cyclodextrin to the semen diluent before freezing resulted in more sperm binding to the zona pellucida, increased the cleavage rate and the development to the blastocyst stage.

In conclusion, the present study provides a novel evidence that, the addition of methyl β cyclodextrin to the semen diluent prior to cryopreservation improved the freezability and in vitro fertilizing potentials of the buffalo semen, in a dose dependent trend, presumably by promoting the cholesterol efflux from the sperm plasma membrane and therefore, increasing plasma membrane fluidity before freezing.

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تأثير إضافة ميثيل بيتا سيكلوديكسترين علي قابليه حيامن الجاموس للتجميد وقدرتها الاخصابيه معمليا

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تتعرض نسبه كبيره من حيامن الجاموس لفقد قدرتها الاخصابيه أثناء عملية التجميد وذلك بسبب تغير في درجة نفاذية الغشاء البلازمي المحيط بالحيامن. وحيث أن الكوليسترول يعتبر من المكونات الأساسية التي تحافظ علي تماسك الغشاء البلازمي للحيامن، وجدت حديثا علاقة طردية بين إخراج الكوليسترول من الغشاء البلازمي المحيط بالحيامن وزيادة قابلية الحيامن للتجميد. وتعتبر مادة ميثيل بيتا سيكلو ديكسترين من أهم المواد التي تعمل علي إخراج الكوليسترول من الغشاء البلازمي المحيط بالحيامن مما يزيد من درجة نفاذيته قبل التجميد و بالتالى يحسن من قابليه الحيامن للتجميد. ولذلك تهدف الدراسة الحاليه الي دراسه تآثيرإضافه ميثيل بيتا سيكلو ديكسترين بتركيزات مختلفه الي مخفف التريس قبل التجميد علي قابليه حيامن الجاموس للتجميد وقدرتها الاخصابيه معمليا. تم تجميع عينات السائل المنوي الجاموسي و تخفيفه في مخفف التريس المضاف إلية تركيزات مختلفة من مادة ميثيل بيتا سيكلو ديكسترين (التجربة الأولى) أو المضاف إليه الكوليسترول-٣- سلفات مع أو بدون التركيز الأمثل لمادة ميثيل بيتا سيكلو ديكسترين (التجربة الثانية) وبعد تبريد وتجميدا لمني الجاموسي بالنظام الفرنسي تم تقيمه من حيث نسبه الحركة الأماميه و معدل الحيويه وتشوهات القلنسوة وكذا قدرته الاخصابيه باستخدام تكنولوجيا الإخصاب المعملي كما تم قياس تركيز الكوليسترول قبل وبعد التجميد. وأظهرت نتائج التجربة الأولى أن مادة ميثيل بيتا سيكلو ديكسترين تحسن من قابليه الحيامن للتجميد طبقا للتركيز المستخدم. حيث أوضحت نتائج الدراسة الحالية أن إضافة ٥ ملليجرام/مللي من مادة ميثيل بيتا سيكلو ديكسترين إلى مخفف التريس قبل التجميد أدي إلى زيادة معنوية كبيره في نسبه الحركة الأماميه بعد الاساله و معدل حيويه المني كما أدي إلى انخفاض معنوي كبير في نسبه تشوهات القلنسوة (٥٧.٥٠%، ١٥٧.٥٠، ١٢.٠٠% علي التوالي) بالمقارنة بالمجموعة الضابطة (٤١.٢٥%، ١٠٥.٥٥، ١٨.٢٥% علي التوالي) كما زاد معدل النمو إلى الطور التوتي وطور البلاستوسيت (٢٤.٤٤%، ١٥.٥٦% علي التوالي) بالمقارنة بالمجموعة الضابطة (٦.٣٨% و٢.١٣% علي التوالي) كما زاد معدل إخراج الكوليسترول من المني بالمقارنة بالمجموعة الضابطة . كما اوضحت نتائج التجربه الثانيه ان إضافه ٥ ملليجرام/مللي من ماده ميثيل بيتا سيكلو ديكسترين الي مخفف التريس قبل التجميد قد قلل الي حد بعيد من التآثير الضار الناتج من إضافه الكوليستيرول-٣- سلفات علي قابليه حيامن الجاموس للتجميد وقدرتها الاخصابيه معمليا. ومن خلال نتائج الدراسه الحاليه يمكن ان نستنتج ان إضافه ٥ ملليجرام/مللي من ماده ميثيل بيتا سيكلو ديكسترين الي مخفف التريس قبل التجميد ادي الي تحسين قابليه حيامن الجاموس للتجميد وكذا قدرتها الاخصابيه معمليا وذلك عن طريق اخراج الكوليستيرول من الغشاء البلازمي للحيامن مما يؤدي الي زياده نفاذيته قبل التجميد.