Global Veterinaria 11 (3): 280-284, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.3.7612

Effect of Addition of Catalase with or without L-Tryptophan on Cryopreservation of Bull Extended Semen and Conception Rate

Reda I. El-sheshtawy, Walid S. El-Nattat and Hussein A. Sabra

Departmant of Animal Reproduction and AI,
National Research Center, Dokki, Egypt

Abstract: The activity of sperm cells after thawing depends on the ratio of reactive oxidant species and antioxidant system. The aim of the present study was to evaluate the effect of catalase (alone or combined with tryptophan as antioxidants) added to the extender on the livability of cryopreserved cattle spermatozoa and on the conception rate of cattle artificially inseminated with these extended semen. Five mature cattle bulls were used as semen donors. Ejaculates were collected using an artificial vagina at weekly intervals for 5 weeks. Tris-citric acid-fructose egg yolk (TCFY) diluent was used as basal control. Catalase (CAT) (25, 50, 75, 100 and 150 U/ml) and CAT (75 U/ml, as best results in vitro) + L-tryptophan (5 mM/L) were added to basal diluent. Seven aliquots of semen were diluted respectively at 37°C with each extender in order to provide concentration of 60 million sperm/ml. Diluted semen filled in 0.25 ml polyvinyl French straws was cryopreserved. Subjective semen characteristics (motility, alive and abnormality %) beside the conception rate in a field test practice were studied. The post-thawing sperm motility significantly ($P<0.003$) improved in CAT supplemented groups (38.33 ± 1.67 (for 25 U/ml), 43.33 ± 1.67, 41.67 ± 1.67 and 40.00 ± 2.89% (for 75, 100 and 150), respectively) as compared to the control group (31.67 ± 1.67%). A significant ($P<0.047$) improvement in CAT + L-tryptophan supplemented group (38.33 ± 1.67) as compared to the control group (31.67 ± 1.67) was found. The higher conception rate was obtained using the concentration of 25 U/ml CAT (78.13%) followed by 50 U/ml (70.37%) as compared to the control group (59.09%). Thence, we concluded that addition of catalase in low concentrations (25, 50 and 75 U/ml) improved semen quality and conception rate of post-thaw semen. However, the use of combination of catalase-tryptophan improved semen quality with no improvement in conception rate.

Key words: Bull · Catalase · Tryptophan · Diluent · Cryopreservation · Conception Rate

INTRODUCTION

The mammalian sperm is an active cell, which spontaneously generate reactive oxygen species (ROS). This production starts as the sperm cells exit the cauda epididymis [1]. ROS in low quantities is essential for sperm motility, acrosome reaction, capacitation and fertilization [2]. ROS including hydrogen peroxide (H_2O_2), superoxide anion O^{2l} and hydroxyl radical (-OH) are strong oxidants and cause sperm dysfunction [3, 4].

Cryopreservation is considered as an important inducer of ROS production [5, 6]. Sperm cells are susceptible to lipid peroxidation as they contain a high content of unsaturated fatty acids in their membranes while, they lack a significant intracytoplasmic antioxidants [7, 8]. Seminal plasma is a powerful source of antioxidants [9]. Natural antioxidants exert a protective effect in preserving the metabolic activity and cellular viability of cryopreserved bovine spermatozoa [10]. Adding catalase to semen extender improved the survival of ram spermatozoa at 5°C [11, 12], post-thawed sperm survival of boar spermatozoa [6] and reduced the deleterious effect of cryopreserved stallion semen [13]. Thus the objective of this study was to evaluate the effect of catalase as an antioxidant added to the semen extender on the livability of cryopreserved cattle spermatozoa and on the conception rate of cattle artificially inseminated with this extended semen.

Corresponding Author: Walid El-Nattat, Departmant of Animal Reproduction and AI,
National Research Center, Dokki, Egypt.

MATERIALS AND METHODS

Semen Collection and Initial Evaluation: Five mature cattle bulls maintained at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used as semen donors. Ejaculates were collected using a bovine adapted artificial vagina at weekly intervals for 5 weeks. Semen samples were initially evaluated for subjective sperm motility and sperm concentration. Ejaculates fulfilling minimum standard of sperm motility (70%) and sperm morphology (80%) were processed for freezing. The ejaculates were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time for 10 minute at 37°C in the water bath before dilution.

Semen Processing: The reference cryopreservation extender (Control) was Tris-citric acid-fructose egg yolk (TCFY) diluent, prepared according to Foote [14].

The Catalase (CAT) and L-tryptophan were obtained from Fluka BioChemika (Switzerland) and Sigma Chemical Co.(USA), respectively and added to the control extender. The concentrations of CAT was 25, 50, 75, 100 and 150 U/ml [11], while L-tryptophan was added at a concentration of 5 mM/L [15, 16] to the best *in vitro* results of CAT. Seven aliquots of semen were diluted at 37°C with the respective extender concentration (control, CAT [25, 50, 75, 100 and 150 U/ml] and CAT[75 U/ml]-L-tryptophan [5 mM/ml]) in order to provide concentration of 60 million sperm/ml. Extended semen was slowly cooled (approximately for 2 hrs) to 4°C and equilibrated for 4 hrs. Semen was packed into 0.25 ml polyvinyl French straws. After equilibrium periods, the straws were horizontally placed on a rack and frozen in a vapour 4 cm above liquid nitrogen for 10 minutes and were then dipped in liquid nitrogen.

Assessment of Semen Quality Parameters: The assessment was undertaken on raw semen, after cooling and after freeze thawing of cattle bull spermatozoa. Frozen straws were thawed at 37°C/ 1 minute. The parameters studied were subjective semen characteristics (motility, alive and abnormality %) beside the conception rate in a field test practice.

Sperm Motility: Sperm motility % was subjectively assessed using microscope set at magnification of 400 and equipped with a heating plate (37°C). Visual motility was microscopically assessed with closed circuit television [17].

Sperm Abnormalities and Viability: This was established by Eiosin/Nigrosin staining [18]. All the semen evaluation was done by single person to avoid individual variations.

Conception Rate (CR): One hundred and nineteen cows were inseminated with extended bull semen to which different concentrations of CAT and twenty six cows were inseminated with extended bull semen to which a combination of CAT+L-tryptophan were added. Forty four cows were inseminated with bull semen diluted with TCFY (Control group). Pregnancy was confirmed by rectal palpation 2 months later after insemination. The inseminated cows were used via the cooperation with the Veterinary Services Organization in Fayoum Governorate. CR was calculated according to the equation:

$$CR = \frac{no.of\ conceived\ cows}{total\ no.of\ inse\min ated\ cows} x100 .$$

Statistical analysis data were analyzed using the SPSS [19] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between additives replications. Number of straws examined represented the replicate (n=30). LSD was calculated for significant variance at $P<0.05$. Student's t-test was calculated for CAT+L-tryptophan combination.

RESULTS

Exp. 1: Data in tables 1 and 2 showed the effects of adding CAT to semen extender on some sperm parameters. Data of tables 3 and 4 showed the effects of adding a combination of CAT and L-tryptophan to semen extender on some sperm parameters. Sperm abnormalities were significantly ($P<0.0001$) improved when CAT was added to the extender at a concentration of 75 U/ml after cooling (13.00 ± 1.00%) as compared to the control value (14.00 ± 1.00%). The post-thawing sperm motility significantly ($P<0.003$) improved in 25, 75, 100 and 150 U/ml CAT supplemented groups (38.33 ± 1.67, 43.33 ± 1.67, 41.67 ± 1.67 and 40.00 ± 2.89%, respectively) as compared to the control group (31.67 ± 1.67%).

The post-thawing sperm abnormalities significantly ($P<0.0001$) improved in 50, 75 and 150 U/ml CAT supplemented groups (18.33 ± 0.33, 15.67 ± 0.33 and 17.67 ± 0.33%, respectively) as compared to the control group (16.00 ± 0.58%).

Table 1: Effect of the addition of catalase (CAT) to the extender on bull semen quality parameters after cooling at 5°C

	Catalase concentration (U/ml)						
Parameter	25	50	75	100	150	Control (TCFY)	Sig
Motile%	88.33 ± 1.67	90.00 ± 2.89	90.00 ± 2.89	90.00 ± 2.89	88.33 ± 1.67	90.00 ± 2.89	0.987
Alive%	85.00 ± 2.89	83.33 ± 3.33	83.33 ± 1.67	86.67 ± 4.41	88.33 ± 3.33	85.00 ± 2.89	0.858
Abnormal%	16.33^{b} ± 0.67	15.67^{b} ± 0.33	13.00^{c} ± 1.00	22.00^{a} ± 1.00	14.33^{bc} ± 0.33	14.00^{bc} ± 1.00	0.000

Within row, means with different alphabetical superscripts are significantly different at least at P<0.05.

Table 2: Effect of the addition of catalase (CAT) to the extender on post-thaw bull semen quality parameters

	Catalase concentration (U/ml)						
Parameter	25	50	75	100	150	Control (TCFY)	Sig
Motile%	38.33^{a} ± 1.67	31.67^{b} ± 1.67	43.33^{a} ± 1.67	41.67^{a} ± 1.67	40.00^{a} ± 2.89	31.67^{b} ± 1.67	0.003
Alive%	66.67 ± 3.33	66.67 ± 3.33	61.67 ± 1.67	61.67 ± 1.67	63.33 ± 3.33	61.67 ± 1.67	0.531
Abnormal%	16.67^{bc} ± 0.88	18.33^{a} ± 0.33	15.67^{c} ± 0.33	16.33^{bc} ± 0.33	17.67^{ab} ± 0.33	16.00^{bc} ± 0.58	0.000

Within row, means with different alphabetical superscripts are significantly different at least at P<0.05.

Table 3: Effect of addition of catalase and tryptophan combination to the extender on bull semen quality after cooling at 5°C

Parameter	CAT+TRYPT.	Control	Sig.
Motility%	86.67 ± 3.33	71.67 ± 1.67	0.02*
Alive%	86.67 ± 1.67	81.67 ± 1.67	0.10
Abnormality%	14.67 ± 0.33	17.33 ± 0.33	0.005*

*Significant

Table 4: Effect of addition of catalase and tryptophan combination to the extender on post-thaw bull semen quality

Parameter	CAT+TRYPT.	Control	Sig.
Motility%	38.33 ± 1.67	31.67 ± 1.67	0.05*
Alive%	63.33 ± 1.67	56.67 ± 3.33	0.148
Abnormality%	17.33 ± 0.88	21.00 ± 1.00	0.05*

*Significant

Table 5: Conception rate (%) of cows inseminated with extended bull semen with different concentrations of catalase or catalase and tryptophan combination

Extender addition	Conception rate %
Control	59.09
Catalase enzyme	
25 µ/ml	78.13
50 µ/ml	70.37
75 µ/ml	59.09
100 µ/ml	52.17
150 µ/ml	53.33
Catalase and tryptophan	
CAT (75 µ/ml) + Trypt. (5 mM/L)	57.69

From these results, it appeared that the addition of CAT in a concentration of 75 U/ml to basal extender gave good results *in vitro*.

Sperm motility significantly (P<0.016) improved in CAT + L-tryptophan supplemented group after cooling (86.67 ± 3.33) as compared to the control (71.67 ± 1.67). Also, sperm abnormalities significantly (P<0.005) decreased (14.67 ± 0.33) as compared to the control (17.33 ± 0.33).

Post-thawing sperm motility significantly (P<0.047) improved in CAT + L-tryptophan supplemented group (38.33 ± 1.67) as compared to the control group (31.67 ± 1.67). Also, sperm abnormalities significantly (P<0.05) improved in CAT + L-tryptophan supplemented group (17.33 ± 0.88) as compared to the control group (21.00 ± 1.00).

Table 5, reveals the conception rate in CAT and CAT+L-tryptophan groups. The higher conception rate was given with the concentration of 25 U/ml CAT (78.13%) followed by 50 U/ml (70.37%) as compared to the control group (59.09%).

DISCUSSION

The application of AI in animal breeding strategies has been shown to have the potential to quickly disseminate genes from the supergenetic males for improving productive traits. The quality of frozen semen is the most influenced factor for conception rate [20]. The composition of the extender in which semen is diluted before freezing is one of the most factors that influence the success of cryopreservation [21].

Cryopreservation of cattle semen often induces an additional source for ROS attack on sperm due to decreased activities of antioxidant enzymes and the sperm membrane became more susceptible to lipid peroxidation [16] which affects the membrane permeability [4]. Addition of CAT as antioxidant acts through avoiding the overproduction of ROS by converting the superoxide anion (O^{2-}) to H_2O_2 [22]. The results of the present study revealed an improvement of semen characteristics and conception rate in CAT supplemented groups and this referred to the scavenger effect of CAT enzyme which participates in the enzymatic metabolism of H_2O_2 producing water and dioxygen besides it prevents the formation of OH, thus reducing the oxidative stress [23]. Also, CAT addition increases the intracellular sperm ATP level [22]. CAT-L-tryptophan supplemented group gave improved sperm motility and abnormalities but no improvement in conception rate. This may be due to the higher concentration of L-tryptophan used where L-amino acid oxidase produced from the dead sperms reacts with L-tryptophan producing the toxic hydrogen peroxide and ammonium ions [24]. This toxic effect occurs during the journey of the sperm in the female genital tract giving low conception rate.

Thence, we concluded that addition of catalase in low concentrations (25, 50 and 75 U/ml) improved semen quality and conception rate of post-thaw semen. However, the use of combination of catalase-tryptophan improved semen quality with no improvement in conception rate.

REFERENCES

1. Aitken, R.J., 2000. Possible redox regulation of sperm motility activation. J. Androl., 21(4): 491-496.
2. Mark, A.B. and R.J. Aitken, 2004. The importance of redox regulated pathways in sperm cell biology. Mol. Cell. Endocrinol., 216(1-2): 47-54.
3. Aitken, R., 1984. A free radicals theory of male infertility. Reproduction, Fertility and Development, 6: 19-24.
4. Awda, B.J., M. Mackenzie-Bell and M.M. Buhr, 2009. Reactive oxygen species and boar sperm function. Biol. Reprod., 81(3): 553-561.
5. Watson, P.F., 2000. Causes of reduced fertility with cryopreserved semen. Anim. Reprod. Sci., 60-61: 481-492.
6. Roca, J., M.J. Rodriguez, M.A. Gil, G. Carvajal, E.M. Garcia, C. Cuello, J.M. Vazquez and E.A. Martinez, 2005. Survival and in vitro fertility of boar spermatozoa frozen in the presence of superoxide dismutase and/or catalase. J. Androl., 26: 15-24.
7. Storey, P.T., 1997. Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. Molecular Human Reproduction, 3: 203-213.
8. Alvarez, J.G. and B.T. Storey, 1989. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation Gamete Research, 23: 77-90.
9. Saleh, R.A. and A. Agarwal, 2002. Oxidative stress and male fertility: from research bench to clinical practice. J. Androl., 23: 737-752.
10. O'Flaherty, C., M. Beconi and N. Beorlegui, 1997. Effect of natural antioxidants, superoxide dismutase and hydrogen peroxide on capacitation of frozenduring thawed bull spermatozoa. Andrologia, 29: 269-275.
11. Maxwell, W.M. and T. Stojanov, 1996. Liquid storage of ram semen in the absence or presence of some antioxidants. Reproduction, Fertility and Development, 8: 1013-1020.
12. Câmara, D.R., M.M.C. Mello-Pinto, I.C. Pinto, O.O. Brasil, J.F. Nunes and M.M.P. Guerr, 2011. Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. Small Rumin. Res., 100: 44-49.
13. Bustamante-Filho, I.C., C.D. Pederzolli, A.M. Sgaravatti, R.C. Mattos, C.S. Dutra-Filho and M.I.M. Jobim, 2006. Activity of glutathione peroxidase and catalase in stallion semen during cryopreservation. Anim. Reprod. Sci., 94: 70-73.
14. Foote, R.H., C.C. Brockett and M.T. Kaproth, 2002. Motility and fertility of bull sperm in whole milk extender containing antioxidants. Anim. Reprod. Sci., 71: 13-23.
15. El-Sheshtawy, R.I., W.S. El-Nattat, H.A. Sabra and Amal H. Ali, 2012. Cryopreservation of extended bull semen usin aromated ad non aromated amino acids with emphasis on conception rate. Global Vet., 9(5): 508-511.

16. El-Sisy, G.A., W.S. El-Nattat and R.I. El-Sheshtawy, 2007. Buffalo semen quality, antioxidants and peroxidation during chilling and cryopreservation. Online J. Vet. Res., 11: 55-61.
17. Graham, E.F., M.K.L. Schmehl and M. Maki-Laurila, 1970. Some physical and chemical methods of evaluating semen. Proceeding of the 3 NAAB Technology Conference of Artificial Insemination and Reproduction, Milwaukee, W.I., National Association of Animal Breeding, Columbia, Mo.
18. Foote, R.H., 1970. Fertility of bull semen at high extension rates in Tris buffered extenders. J. Dairy Sci., 53: 1475-1477.
19. SPSS, 2005. SPSS 14.0 for WindowsEvaluation Version Release 14.0.0
20. Saacke, R.G., S. Nadir and R.L. Nebel, 1994. Relationship of semen quality to sperm quality, fertilization and embryo quality in ruminants. Theriogenology, 40: 1207-1214.
21. Woelders, H. and A.P. Malva, 1998. How important is the cooling rate in cryopreservation of (bull) semen and what is its relation to thawing rate and glycerol concentration. Reprod. Dom. Anim., pp: 33.
22. Bilodeau, J.F., S. Blanchette, N. Cormier and M.A. Sirard, 2002. Reactive oxygen species-mediated loss of bovine sperm motility in egg yolk Tris extender: protection by pyruvate, metal chelators and bovine liver or oviductal fluid catalase. Theriogenology, 57: 1105-1122.
23. Ondei, L.S., L.M. Silveira, A.A. Leite, D.R.S. Souza, A.A.S. Pinhel, S. Percario, O. Ricci and C.R. Bonini-Domingos, 2009. Lipid peroxidation and antioxantioxidant capacity of G6PD-deficient patients with A-(202G>A) mutation. Genet. Mol. Res., 8(4): 1345-1351.
24. Macmillan, K.L., J.L. Tiku and N.L. Hart, 1972. Toxic effects of aromatic amino acids on the livability of bull spermatozoa. Aust. J. Biol. Sci., 25: 1039-1045.