Tropical Animal Health and Production (2021) 53: 238
https://doi.org/10.1007/s11250-021-02668-5

REGULAR ARTICLES

Effect of Tris-extender supplemented with a combination of turmeric and ethylene glycol on buffalo bull semen freezability and in vivo fertility

Reda I. El-Sheshtawy[1]

Received: 14 July 2020 / Accepted: 15 March 2021 / Published online: 1 April 2021

Abstract

The objective of this study was to evaluate the effect of Tris-extender supplemented with a combination of turmeric and ethylene glycol on buffalo bull semen preservability and in vivo fertility. Five tubes (each contain 5 ml Tris-citric acid-fructose yolk, TCFY). The first tube contains turmeric extract and ethylene glycol and kept as a control. The other four tubes contain 1.5% ethylene glycol plus turmeric extract as follows: 100 μl/5 ml, 200 μl/5 ml, 300 μl/5 ml, and 400 μl/5 ml *v*/*v*). Pooled bull semen was added to the Tris extender and to Tris turmeric ethylene glycol. Extended semen was subjected to semen freezing protocol. Semen assessment was carried out. In Tris turmeric ethylene glycol post-cooling, sperm motility and alive sperms were significantly higher in the first concentration of Tris turmeric ethylene glycol (TTE_1), sperm abnormalities were significantly lower in TTE_1 and TTE_2, and sperm membrane integrity (HOST) was significantly higher in TTE_1 and acrosome percent was significantly higher in TTE_1, TTE_2, and TTE_4 if compared to the control. Post-thawing, sperm motility was significantly higher in TTE_1 as compared to the control and other concentrations of TE. All concentrations of TE were significantly higher in sperm membrane integrity (HOST) as relative to the control. Alive sperms were kept in TTE_1 as the control. Acrosome percent was kept in most concentrations as the control. It is concluded that, in cooled and post-thawed semen, the first concentration (TTE_1) gave the best sperm quality and conception rate.

Keywords Buffalo · Semen · Freezing · Turmeric · Ethylene glycol

Introduction

Artificial insemination (AI) is considered the main tool for dissemination of the supergenetic characters to improve the genetic constitution of the livestock (Vishwanath and Shannon 2000; Kubkomawa et al. 2018).

Cryopreservation causes damage of about 50% of the stored spermatozoa (Watson 2000) mainly due to the intracellular ice crystallization during freezing (Watson 2000; Akhter et al. 2008). The freeze-thaw process results in structural and functional damages caused by overaccumulation of reactive oxygen species (ROS) (Guthrie and Welch 2006). The spermatozoa membrane is susceptible to lipid peroxidation due to its polyunsaturated fatty acid content which leads to oxidative damage with subsequent reduced motility, viability, and DNA integrity (Nair et al. 2006; Andrabi 2009). So the composition of the extender is of a great importance to minimize such damage (Curry et al. 1994; Andrabi 2009). The extenders used for semen preservation of domestic species must have adequate pH and buffering capacity, suitable osmolality, and should protect sperm cells from cryogenic injury (Salamon and Maxwell 2000; Barbas and Mascarenhas 2009). Ethylene glycol has fewer hazardous effect on viability, motility, and acrosome status if compared to glycerol (Ball and Vo 2001).

Improvement of semen cryopreservation of the buffalo bulls is a great objective; this could be achieved through supplementation of the extended semen with antioxidants. Plant extracts are considered a major category to fulfill this purpose. Turmeric extract contain curcumin which is a main ingredient acting as antioxidant in semen extenders (Petruska et al.

This work was carried out in Abassia Buffalo Semen Freezing Center, Central Organization for Veterinary Services, Ministry of Agriculture, Egypt and National Research Centre, Giza, Egypt.

✉ Reda I. El-Sheshtawy
rielsheshtawy@gmail.com

[1] Animal Reproduction and Artificial Insemination Department, National Research Centre, Dokki, Giza, Egypt

2014). Curcumin is the principal of curcuminoid of turmeric (*Curcuma longa*), a member of ginger family. Curcuminoids are natural phenols responsible for turmeric yellow color (Nelson et al. 2017). Turmeric extract contain curcumin with other curcuminoids and essential oils which were found to be bioactive (Kulkarni et al. 2012).

Turmeric is a useful plant. Curcumin is a phytochemical having antioxidant and anti-inflammatory effect and is extracted from the rhizome of turmeric longa. Curcumin is demonstrated to have a protective effect for spermatozoa in vitro depending on its concentration where low concentrations improved sperm motility while high concentrations decreased sperm motility (Głombik et al. 2014). Curcumin is a polyphenolic insoluble in water that scavenges free radicals (Sharma 1976) through decreasing generation of reactive oxygen species (ROS), as H_2O_2 and nitrite. Addition of curcumin to fresh bull semen significantly increased sperm output after thawing (Bucak et al. 2011). Administration of curcumin to male rodents improved testicular function and fertility (Sahoo et al. 2008; Mathuria and Verma 2008). So, the objective of this study was to evaluate the effect of Tris-extender supplemented with a combination of turmeric and ethylene glycol on buffalo bull semen preservability and in vivo fertility.

Material and methods

Preparation of different semen extenders

Tris base extender: Tris-citric acid-fructose diluent (TCF) with 20% whole egg yolk was prepared (de Paz et al. 2010). TCF ingredients are 3.029 g Tris, 1.679 g citric acid monohydrate, 1.259 g fructose, and glycerol 6.4% in 100 ml distilled H_2O.

Preparation of turmeric extract: 4 g turmeric powder + 60 ml ethanol in a test tube. 4 g turmeric powder + 60 ml distilled water in another tube. Using stirrer for mixing in each tube, filtration. The filtrate is left at 40 °C for 24 h till evaporation. The residues in both tubes were mixed together and dissolved in 2 ml Tris and kept as a stock solution from which the different concentrations were made.

Turmeric ethylene glycol enriched extender [TEEE]: five tubes (each contain 5 ml TCFY). The first tube contains turmeric extract and ethylene glycol and kept as a control. The other four tubes contain 1.5% ethylene glycol plus turmeric extract (from the stock solution) as follows: 100 μl/5 ml, 200 μl/5 ml, 300 μl/5 ml, and 400 μl/5 ml *v*/*v*.

Semen collection and initial evaluation

Semen from five mature buffalo bulls kept at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used. Ejaculates were collected using artificial vagina at weekly intervals for 18 weeks. Semen samples were initially evaluated for subjective sperm motility and sperm concentration. Ejaculates fulfilling minimum sperm motility (70%) and normal sperm morphology were pooled in order to have sufficient semen for a replicate and to exclude the bull effect. Semen was hold for 10 min at 37 °C in the water bath before dilution.

Semen processing

Semen samples were diluted with TCFY extender and used as control and other aliquots of pooled semen samples were diluted with TCFY extenders containing the different concentrations of turmeric extract to reach concentration of 60 million sperm/ml. Extended semen was cooled slowly (approximately for 2 h) to 5 °C and equilibrated for 2 h. Semen was packed into 0.25-ml polyvinyl French straws. After this period, the straws were placed horizontally on a rack and frozen in vapor 4 cm above liquid nitrogen for 10 min and were then plunged in liquid nitrogen.

Evaluation of semen quality parameters

The assessment was implemented post-cooling and on freeze-thawed bull spermatozoa. Frozen straws were thawed at 37 °C/1 min. The parameters studied were subjective semen characteristics (motility, alive, abnormality, hypoosmotic swelling test (HOST), and acrosome status) (Table 1).

Sperm motility

Sperm motility was assessed (Graham et al. 1970).

Sperm membrane integrity

Plasma membrane integrity of buffalo bull spermatozoa was assessed using the hypo-osmotic swelling test (HOST) (Jeyendran et al. 1984; Ahmad et al. 2003).

Percentage of live sperm and normal acrosome

The dual staining procedure with trypan blue-Giemsa stain was performed (Salisbury et al. 1978).

Sperm morphology

Abnormal sperm percent was examined in eosin nigrosine stained semen smears (Gormier et al. 1997).

Statistical analysis

Data were analyzed using the SPSS (2005) computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between control and additive

Table 1 Effect of Tris extender enriched with combination of turmeric ethylene glycol on post-cooling buffalo semen quality (mean ± SE)

Diluent	Motility	Alive	Abnormalities	HOST	Acrosome
TTE_1	96.66 ± 1.66^{c}	91.00 ± 1.00^{c}	$7.00 \pm .57^{a}$	87.98 ± 1.05^{a}	$85.33 \pm .33^{b}$
TTE_2	$90.00 \pm .00^{ab}$	86.00 ± 1.00^{b}	$7.33 \pm .33^{a}$	80.54 ± 3.28^{b}	$80.66 \pm .66^{a}$
TTE_3	88.33 ± 1.66^{a}	81.33 ± 1.33^{a}	$11.33 \pm .33^{b}$	$84.31 \pm .94^{b}$	82.66 ± 1.45^{ab}
TTE_4	88.33 ± 4.40^{a}	81.33 ± 1.33^{a}	$11.33 \pm .33^{b}$	62.65 ± 7.57^{b}	$84.33 \pm .66^{b}$
Control	88.33 ± 1.66^{a}	85.66 ± 1.20^{b}	18.33 ± 1.66^{c}	$80.73 \pm .76^{b}$	$80.00 \pm .00^{a}$
Total	90.33 ± 1.24	85.06 ± 1.05	11.06 ± 1.13	79.24 ± 2.73	82.78 ± .63
P value	.125	.001	.000	.007	.009

Means bearing different superscripts between different extenders and differ at 5% and 1% levels of probability

TCFYG control Tris-citrate-fructose-egg yolk-glycerol, *TTE_1* Tris TE_1, *TTE_2* Tris TE_2, *TTE_3* Tris TE_3, *TTE_4* Tris TE_4

replications. Significant difference between means was calculated using Duncan test at $P < 0.05$.

In vivo fertility rate (CR)

Number of buffalo females ($n = 320$) were inseminated with the TTE post-thawed semen and with the post-thawed semen extended in TCFY (control group) (Table 2). Pregnancy was recorded by rectal palpation after 2 months from insemination. The inseminated cows were used via the cooperation in Beni-Suef Governorate. CR was computed according to the equation:

$$\mathrm{CR} = \frac{\text{no.of conceived buffaloes}}{\text{total no.of inseminated buffaloes}} \times 100$$

Results

In Tris turmeric ethylene glycol post-cooling, sperm motility and alive sperms were significantly higher in TTE_1, sperm abnormalities were significantly ($P < 0.000$) lower in TTE_1 and TTE_2, and sperm membrane integrity (HOST) was significantly ($P < .007$) higher in TTE_1 and acrosome percent was significantly ($P < .009$) higher in TTE_1, TTE_2, and TTE_4 if compared to the control. Post-thawing, sperm motility was significantly ($P < 0.000$) higher in TTE_1 as compared to the control and other concentrations of TE. All concentrations of TE were significantly ($P < .004$) higher in sperm membrane integrity (HOST) as relative to the control. Alive sperms were kept in TTE_1 as the control. Acrosome percent was kept in most concentrations as the control. The conception rate was the best in TTE_1 (Table 3).

Discussion

Several factors have been reported to influence the cryosurvival of spermatozoa including osmotic stress, ice crystal formation, toxicity of the cryoprotectants, and the individual variability (Neild et al. 2003; Ferrusola et al. 2009). Among various causes, oxidative stress has been reported to affect the fertility and physiology of frozen/thawed spermatozoa (Agarwal et al. 2008; O'Flaherty 2014; Smith et al. 2006). Oxidative stress occurs as a consequence of imbalance between the levels of reactive oxygen species (ROS) production and the antioxidant capacity of the cell (Halliwell 2006). Excessive amounts of ROS are harmful to the sperm cells (Halliwell and Gutteridge 2007); low level of these molecules are required to induce sperm capacitation in human, a process

Table 2 Effect of Tris extender enriched with combination of turmeric ethylene glycol on the post-thawed extended buffalo bull semen (mean ± SE)

Diluent	Motility	Alive	Abnormalities	HOST	Acrosome
TTE_1	61.66 ± 1.66^{d}	82.33 ± 2.33^{c}	$10.33 \pm .33^{b}$	75.00 ± 1.78^{b}	70.00 ± 2.64^{c}
TTE_2	25.00 ± 2.88^{b}	69.00 ± 2.08^{b}	$15.00 \pm .57^{c}$	72.81 ± 1.01^{b}	52.33 ± 2.33^{a}
TTE_3	21.66 ± 1.66^{b}	72.00 ± 2.51^{b}	$20.33 \pm .33^{e}$	70.44 ± 3.34^{b}	60.33 ± 3.17^{ab}
TTE_4	8.33 ± 1.66^{a}	$60.33 \pm .33^{a}$	$17.66 \pm .33^{d}$	72.62 ± 1.94^{b}	61.00 ± 3.78^{abc}
Control	43.33 ± 3.33^{c}	86.66 ± 3.33^{c}	$6.66 \pm .33^{a}$	48.66 ± 7.51^{a}	76.33 ± 6.83^{bc}
Total	32.00 ± 5.04	74.06 ± 2.67	14.00 ± 1.32	67.91 ± 2.98	64.00 ± 2.70
P value	.000	.000	.000	.004	.009

Means bearing different superscripts between different extenders and differ at 5% and 1% levels of probability

TCFYG control Tris-citrate-fructose-egg yolk-glycerol, *TTE_1* Tris TE_1, *TTE_2* Tris TE_2, *TTE_3* Tris TE_3, *TTE_4* Tris TE_4

Table 3 Effect of Tris extender enriched with combination of turmeric and ethylene glycol on a field conception rate test in buffalo

Treatment	In vivo fertility rate (CR, %)
TTE_1	68%
TTE_2	42%
TTE_3	36%
TTE_4	12%
Control (TCFYG)	53%

that is required for the spermatozoa to acquire their fertilizing ability (O'Flaherty et al. 2003). Under oxidative stress, spermatozoa suffer extensive damage such as peroxidation of membrane lipids, DNA fragmentation (Barroso et al. 2000), low mitochondrial membrane activity (Gallon et al. 2006; Koppers et al. 2008), and inactivation of enzymes associated with motility (de Lamirande and Gagnon 1992).

A variety of antioxidants are represented in the spermatozoa and seminal plasma as represented by antioxidant enzymes (SOD, CAT, and GSH). Their antioxidant capacity is insufficient and gradually declines on extending the freezing process, so antioxidant supplements should be added to the semen extender (Bilodeau et al. 2001).

Recently, there is a great worldwide interest with the beneficial synergistic effects of natural supplements and their multiple ingredients as compared to the single active fractions (Seeram et al. 2004). Semen freezing causes damage to spermatozoa leading to reduction in semen quality (Watson 2000), but it is essential to conserve the supergenetic characters of our local breeds of buffalo. Semen freezing is associated with cryodamage caused by overproduction of oxygen free radicals (Agarwal et al. 2005), so the natural additive to the extender ameliorates the antioxidant effect and consequently improving the fertilizing capacity of frozen spermatozoa (Gadea et al. 2007). The results of the current study, in Tris turmeric ethylene glycol post-cooling, sperm motility, alive sperms, and sperm membrane integrity (HOST), were significantly higher in TTE_1. Sperm abnormalities were significantly lower in TTE_1 and TTE_2. Acrosome percent was significantly higher in TTE_1, TTE_2, and TTE_4 if compared to the control. Post-thawing, sperm motility was significantly higher in TTE_1 as compared to the control and other concentrations of TE. All concentrations of TE were significantly higher in sperm membrane integrity (HOST) as relative to the control. Alive sperms were kept in TTE_1 as the control. Acrosome percent was kept in most concentrations as the control. The conception rate was the best in TTE_1 and this coincides with the best sperm motility at this concentration. These results come in accordance with Bucak et al. (2011) in bovine semen and with those of Mahmoud et al. (2013) who showed that motility may be an applicant indicator for semen characteristics, adding that significant correlations were found between motility and both sperm abnormalities and membrane integrity. Ramos and Wetzels (2001) reported that motility may be a related to DNA status of the sperm cells. Vale (1997) recorded a pregnancy rate over than 50% as a good consequence after AI with post-thawed frozen semen in buffalo. Al Naib et al. (2011) categorized bulls with pregnancy rate of about 50% to be highly fertility, and the sperm of high-fertility bulls is highly efficient in penetrating artificial mucus and to have high potentiality to fertilize oocyte in vitro. Curcumin is the major extract of turmeric; it is a lipophilic polyphenol insoluble in water and scavenge free radicals, significantly inhibiting the generation of ROS (Petruska et al. 2014). Curcumin significantly increase the sperm content of GSH, thus improving the antioxidant capacity of the semen extender (Bucak et al. 2011). Curcumin shows antioxidant activity through binding with egg and soy phosphatidyl choline which in turn binds divalent metal ions and has antibacterial and antiviral effects (Bhowmik et al. 2009). The antioxidant effect of curcumin is referred to its unique conjugated structure which includes two methoxylated phenols and an enol form of b-diketone; this structure revealed ideal free radical trapping ability as a chain breaking antioxidant (Bagchi 2012). Turmeric contains essential oils. The polyunsaturated fatty acids in the essential oils interact with sperm membrane rendering it more stable and resistant to cold shock during cryopreservation, thus improving sperm motility, viability, and sperm membrane integrity (Singh et al. 2012).

Ethylene glycol improved post-thaw sperm characteristics in bull semen (Guthrie et al. 2002), in ram (Silva et al. 2012), in stallion (Mantovani et al. 2002; Alvarenga et al. 2000; Henry et al. 2002), and in buffalo (Swelum et al. 2011). Ethylene glycol has a lower molecular weight and a lower toxicity and higher permeability to spermatozoa if compared to glycerol (Soares et al. 2002; Massip 2001) with reduction of the sperm osmotic stress during cooling and freezing (Gilmore et al. 1995).

However, Büyükleblebici et al. (2014) recorded no improvement of post-thaw sperm motility in bulls on the use of ethylene glycol as a cryoprotectant. It could be concluded that, in cooled and post-thawed semen, the first concentration (TTE_1) gave the best sperm quality and conception rate.

Acknowledgements This work was supported by the National Research Centre (grant number: 10120801). The authors are extremely grateful to the National Research Centre and the Buffalo Semen Freezing Center, Ministry of Agriculture. Also appreciating are due to the team assistants in the article for their cooperation.

Declarations

Ethical approval The experimental design was approved by the Medical Research Ethics Committee of the National Research Centre, Dokki, Egypt, and its registration number is 19/104 and its date is 10/10/2019.

Conflict of interest The author declares no competing interests.

References

Agarwal, A., Prahakaran, S.A. and Said, T.M. 2005 Prevention of oxidative stress injury to sperm. Journal of Andrology, **26**, 654–660.

Agarwal, A., Makker, K. and Sharma, R. 2008. Clinical relevance of oxidative stress in male factor infertility. American Journal of Reproductive Immunology, **59**, 2–11.

Ahmad, Z., Anzar, M., Shahab, M., Ahmad, N. and Andrabi, S.M.H. 2003. Sephadex and sephadex-ion exchange filtration improves the quality and freezability of low-grade buffalo ejaculates. Theriogenology, **59**, 1189–1202.

Akhter, S., Ansari, M.S., Andrabi, S.M.H., Ullah, N. and Qayyum, M. 2008. Effect of antibiotics in extender on bacterial and spermatozoal quality of cooled buffalo (*Bubalus bubalis*) bull semen. Reproduction in Domestic Animals, **43**, 272–278.

Al Naib, A., Hanarahan, J.P., Lonergan, P. and Fair, S. 2011. In vitro assessment of sperm from bulls of high and low fertility. Theriogenology. **76**, 161–167.

Alvarenga, M.A., Landim-Alvarenga, F.C., Moreira, R.M. and Cesarino, M.M. 2000. Acrosomal ultrastructure of stallion spermatozoa cryopreserved with ethylene glycol using two packaging systems. Equine Veterinary Journal, **32**, 541–545.

Andrabi, S.M.H. 2009. Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. Reproduction in Domestic Animals, **44**, 552–569.

Bagchi, A. 2012. Extraction of curcumin IOSR. Journal of Environmental Science, Toxicology and Food Technology, **1**, 1–16.

Ball, B.A., and Vo, A. 2001. Osmotic tolerance of equine spermatozoa and the effects of soluble cryoprotectants on equine sperm motility, viability, and mitochondrial membrane potential. Journal of Andrology, **22**, 1061–9.

Barbas, J.P. and Mascarenhas, R.D. 2009. Cryopreservation of domestic animal sperm cells. Cell and Tissue Banking **10**, 49–62.

Barroso, G., Morshedi, M. and Oehninger, S. 2000. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa. Human Reproduction, **15**, 1338–1344.

Bhowmik, D., Chiranjib, K. P., Kumar, S., Chandira, M. and Jayakar, B. 2009. Turmeric: a herbal and traditional medicine. Archives of Applied Science Research, **1**, 86–108.

Bilodeau, J.F., Blanchette, S., Gagnon, I.C. and Sirard, M.A. 2001. Thiols prevent H_2O_2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology, **56**, 275–286.

Bucak, M.N., Baspinar, N., Tuncer, P.B., Coyan, K., Sariozkan, S., Akalin P.P., Buyukleblebici, S. and Kucukgunay, S. 2011. Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. Andrologia, **44**, 102–109.

Büyükleblebici, S., Tuncer, P.B., Bucak, M.N., Taşdemir, U., Eken, A. , Büyükleblebici, O., Durmaz, E., Sarıözkan, S. and Endirlik, B.Ü. 2014. Comparing ethylene glycol with glycerol and with or without dithiothreitol and sucrose for cryopreservation of bull semen in egg-yolk containing extenders. Cryobiology, **69**, 74–78.

Curry, M.R., Millar, J.D. and Watson, P.F. 1994. Calculated optimal cooling rates for ram and human sperm cryopreservation fail to conform with empirical observations. Biology of Reproduction, **51**, 1014–21.

de Lamirande, E. and Gagnon, C. 1992. Reactive oxygen species and human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes. Journal of Andrology, **13**, 368–378.

de Paz, P., Esteso, M.C., Alvarez, M., Mata, M., Chamorro, C.A. and Anel, L. 2010. Development of extender based on soybean lecithin for its application in liquid ram semen. Theriogenology, **74**, 663–671.

Ferrusola, C. O., Gonzalez Fernandez, L., Macias Garcia, B., Salazar-Sandoval, C., Morillo Rodriguez, A., Rodriguez Martinez, H., Tapia, J.A. and Pena, F.J. 2009. Effect of cryopreservation on nitric oxide production by stallion spermatozoa. Biology of Reproduction, **81**, 1106–1111.

Gadea, J., Gumbo, D., Cánovas, S., García-Vázquez, F.A., Grullon, L. A. and Gardon, J.C. 2007. Supplementation of the dilution medium after thawing with reduced glutathione improves function and the in vitro fertilizing ability of frozen-thawed bull spermatozoa. International Journal of Andrology, **31**, 40–9.

Gallon, F., Marchetti, C., Jouy, N. and Marchetti, P. 2006. The functionality of mitochondria differentiates human spermatozoa with high and low fertilizing capability. Fertility and Sterility, **86**, 1526–1530.

Gilmore, J.A., McGann, L.E., Liu, J., Gao, D.Y., Peter, A.T., Kleinhans, F.W. and Critser, J.K. 1995. Effect of cryoprotectant solutes on water permeability of human spermatozoa. Biology of Reproduction, **53**, 985–95.

Głombik, K., Basta-Kaim, A., Sikora-Polaczek, M., Kubera, M., Starowicz, G. and Styrna, J. 2014. Curcumin influences semen quality parameters and reverses the di(2-ethylhexyl)phthalate (DEHP)-induced testicular damage in mice. Pharmacological Reports, **66**, 782–787.

Gormier, N., Sirard, M.A. and Baile, J.L. 1997. Premature capacitation of bovine spermatozoa is initiated by cryopreservation. Journal of Andrology, **18**, 461–468.

Graham, E.F., Schmehl, M.K.L. and Maki-Laurila, M. 1970. Some physical and chemical methods of evaluating semen. In: Proceeding 3rd NAAB Technology Conference Artificial Insemination Reproduction 12–14 April Milwaukee, WI. National Association of Animal Breeders, Columbia, MO; p. 44–48.

Guthrie, H.D. and Welch, G.R. 2006. Determination of intracellular reactive oxygen species and high mitochondrial membrane potential in Percoll-treated viable boar sperm using fluorescence-activated flow cytometry. Journal of Animal Science, 84, 2089–2100.

Guthrie, H.D., Liu, J. and Critser, J.K. 2002. Osmotic tolerance limits and effects of cryoprotectants on motility of bovine spermatozoa. Biology of Reproduction, **67**, 1811–1816.

Halliwell, B. 2006. Oxidative stress and neurodegeneration: where are we now? Journal of Neurochemistry, **97**, 1634–1658.

Halliwell, B. and Gutteridge, J. 2007. Free radicals in biology and medicine, Press New York. Oxford University.

Henry, M., Snoeck, P.P.N. and Cottorello, A.C.P. 2002. Post-thaw spermatozoa plasma integrity and motility of stallion semen frozen with different cryoprotectants. Theriogenology, **58**, 245–248.

Jeyendran, R.S., Van der Van, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. Journal of Reproduction and Fertility, **70**, 219–228.

Koppers, A.J., De Iuliis, G.N., Finnie, J.M., McLaughlin, E.A. and Aitken, R.J. 2008. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. The Journal of Clinical Endocrinology & Metabolism, **93**, 3199–3207.

Kubkomawa, H.I., Adamu, S.M., Achonwa, C.C., Adewuyi, K.A. and Okoli, I.C. 2018. Beef production and marketing in Nigeria: entrepreneurship in animal agriculture. International Journal of Veterinary Sciences and Animal Husbandry, **3**, 26-40.

Kulkarni, S.J., Maske, K.N., Budre, M.P. and Mahajan, R.P. 2012. Extraction and purification of curcuminoids from turmeric (*Curcuma longa* L.). International Journal of Pharmacology and Pharmaceutical Technology, **1**, 2277–3436.

Mahmoud, K.G.M, EL Sokary, A.A.E., Abou el Roos, M.E.A., Abdel Ghafar, A.D. and Nawito, M. 2013. Sperm characteristics in

cryopreserved buffalo bull semen and field fertility. Iranian Journal of Applied Animal Science, **3**, 777–783.

Mantovani, R., Rota, A., Falomo, M.E., Bailoni, L. and Vincenti L. 2002. Comparison between glycerol and ethylene glycol for the cryopreservation of equine spermatozoa: sperm quality assessment with standard analyses and with the hypoosmotic swelling test. Reproduction Nutrition Development, **42**, 217–26.

Massip, A. 2001. Cryopreservation of embryos of farm animals. Reproduction in Domestic Animals, **36**, 49–55.

Mathuria, N. and Verma, R.J. 2008. Ameliorative effect of curcumin on aflatoxin-induced toxicity in serum of mice. Acta poloniae pharmaceutica, **65**, 339–343.

Nair, S.J., Brar, A.S., Ahuja, C.S., Sangha, S.P. and Chaudhary, K.C.A. 2006. Comparative study on lipid peroxidation, activities of antioxidant enzymes and viability of cattle and buffalo bull spermatozoa during storage at refrigeration temperature. Animal Reproduction Science, **96**, 21–29.

Neild, D.M., Gadella, B.M., Chaves, M.G., Miragaya, M.H., Colenbrander, B. and Aguero, A. 2003. Membrane changes during different stages of a freeze-thaw protocol for equine semen cryopreservation. Theriogenology **59**, 1693–1705.

Nelson, K.M., Dahlin, J., Bisson, J., Graham, J., Pauli, G.F. and Walters M.A. 2017. The essential medicinal chemistry of curcumin. Journal of Medicinal Chemistry, **60**, 1620–1673.

O'Flaherty, C. (2014). Iatrogenic genetic damage of spermatozoa, in: E. Baldi, M. Muratori (Eds.) Genetic damage in human spermatozoa, Springer New York, pp. 117–135.

O'Flaherty, C., Beorlegui, N. and Beconi, M.T. 2003. Participation of superoxide anion in the capacitation of cryopreserved bovine sperm. International Journal of Andrology, **26**, 109–114.

Petruska, P., Marcela Capcarova, M. and Sutovsky, P. 2014. Antioxidant supplementation and purification of semen for improved artificial insemination in livestock species. Turkish Journal of Veterinary and Animal Sciences, **38**, 643–652.

Ramos, L. and Wetzels, A.M. 2001. Low rates of DNA fragmentation in selected motile human spermatozoa assessed by the TUNEL assay. Human Reproduction, **16**, 1703–1707

Sahoo, D.K., Roy, A. & Chainy, G.B. (2008). Protective effects of vitamin E and curcumin on L-thyroxine-induced rat testicular oxidative stress. Chemico-Biological Interactions, **176**(2-3), 121–128.

Salamon, S. and Maxwell, W.M. 2000. Storage of ram semen. Animal Reproduction Science, **62**, 77–111.

Salisbury, G.W., VanDemark, N.L. and Lodge, J.R. 1978. Semen evaluation: In "Physiology of Reproduction and Artificial Insemination of Cattle." 2nd edition. W.H. Freeman & Company, San Francisco, USA.; pp. 400–427.

Seeram, N.P., Adams, L.S., Hardy, M.L. and Heber, D. 2004. Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects. Journal of Agricultural and Food Chemistry, **52**, 2512–2517.

Sharma, O.P. 1976. Antioxidant activity of curcumin and related compounds. Biochem Pharmacol, **25**, 1811–1812.

Silva, R. E.C.B., Cajueiroa, J.F.P., Silva, S.V., Vidala, A.H., Soaresb, P.C. and Guerra M.M.P. 2012. In vitro evaluation of ram sperm frozen with glycerol, ethylene glycol or acetamide. Animal Reproduction Science, 132(3-4), 155–158.

Singh, A.K., Singh, V.K., Narwade, B.M., Mohanty, T.K. and Atreja S.K. 2012. Comparative quality assessment of buffalo (*Bubalus bubalis*) semen chilled (5 °C) in egg yolk-and soya milk-based extenders. Reproduction in Domestic Animals, **47**, 596–600.

Smith, R., Kaune, H., Parodi, D., Madariaga, M., Rios, R., Morales, I. Castro, A. 2006. Increased sperm DNA damage in patients with varicocele: relationship with seminal oxidative stress. Human Reproduction, **21**, 986–993.

Soares, M.P., Rossi, C.A.R., Mezzalira, A. and Cecim, M. 2002. Ethylene glycol on canine semen cryopreservation. Ciencia Rural, **32**, 649–55.

SPSS. SPSS v.14.0 for Windows Evaluation Version Release. 14.0.0, 2005.

Swelum, A.A., Mansour, H.A., Elsayed, A.A. and Amer, H.A. 2011. Comparing ethylene glycol with glycerol for cryopreservation of buffalo bull semen in egg-yolk containing extenders. Theriogenology, **76**, 833–842.

Vale, W.G. 1997. Sperm cryopreservation. *Bubalus bubalis* 1

Vishwanath, R. and Shannon, P. 2000. Storage of bovine semen in liquid and frozen state. Animal Reproduction Science, **62**, 23–53.

Watson, P.F. 2000. The causes of reduced fertility with cryopreserved semen. Animal Reproduction Science, **60–61**, 481–492.