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Research Article

Assessment of Cattle Bull Semen Preservability Using Tris Extender Enriched with Wheat Germ Extract

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Received: August 17, 2020; **Published:** September 15, 2020

Abstract

The Objective was to evaluate the effect of tris-extender supplemented with different concentrations of the wheat germ extract (TW) on buffalo bull semen preservability. Pooled bull semen were extended with Tris extender. Five tubes were prepared. The first tube contains 0 Wheat germ extract in 4 ml tris extender and kept as a control. The other four tubes contain Wheat germ extract as follows (250 µl stock/3.75 ml tris, 500 µl/3.5 ml, 1000 µl/3 ml and 2000 µl/2 ml) and final sperm concentration 60 × 10^6/mL was attained. Aliquots of extended semen (500 µl/3.5 ml, TW2) were subjected to chilling. Extended semen was exposed to freezing protocol. Semen assessment was carried out. Post-cooling results revealed improvement in sperm quality relative to the control. Post-thawing findings showed significant improvement in sperm motility in TW_1, TW_2 TW_3 if compared to TW4 and the control. Alive sperm percent was significantly higher in TW_1, TW_2 and TW_3 if compared to TW_4 and the control. Sperm abnormalities was significantly lower in TW_1, TW2, TW_3 if compared to TW_4. Sperm membrane integrity (HOST) were significantly higher in TW_1, TW_2 if compared to TW3, TW4 and the control. It is concluded that, all concentrations of the wheat germ extract gave good post-cooling except the fourth concentration (TW_4). Post-thawing sperm parameters exhibited that sperm quality was superior in TW_1, TW_2, TW_3 and deteriorated with the concentration (TW_4). Conception rate was the best in TW_1 followed by TW_2 and TW3and the lowest was in TW_4 and the control.

Keywords: *Cattle; Wheat Germ Extract; Semen; Tris; Cryopreservation*

Abbreviations

TW: Tris Wheat Germ Extract; PUFAs: Polyunsaturated Fatty Acids; ROS: Reactive Oxygen Species; TCF: Tris-Citric Acid-Fructose; TCFY: Tris-Citric Acid-Fructose Yolk; HOST: Hyposmotic Swelling Test; CR: Conception Rate

Introduction

Phospholipid composition of sperm, in particular its high content in polyunsaturated fatty acids (PUFAs), maintained plasma membrane fluidity and integrity with subsequent effect on sperm fertility [1]. The differences in species susceptibility of spermatozoa to cooling, freezing, and thawing process seems to be largely attributable to the PUFA contents of sperm plasma membrane [1,2]. The plasma membrane of different mammalian species contains about 70% phospholipids [3].

The freeze-thaw process results in structural and functional damages caused by over accumulation of reactive oxygen species (ROS) [4]. The spermatozoa membrane is susceptible to lipid peroxidation due to its polyunsaturated fatty acids content which leads to oxidative damage with subsequent reduced motility, viability and DNA integrity [5,6]. The success of cryopreservation depends not only on preserving the motility of the spermatozoa but also on maintaining their metabolic function [7]. Commonly, buffalo semen is preserved in milk [8-11], tris-egg yolk [12,13] and egg yolk-citrate [14,15] diluents. These diluents contain additives of animal source (egg yolk and/or milk) which may pose an extreme hazard of microbial contaminants [16,17]. This sanitary risk may reduce fertility of frozen semen directly through producing hazardous metabolites and toxins which deteriorates the semen characteristics, or indirectly through local infection leading to abortion [18,19].

Natural antioxidants have a protective effect preserving the viability and metabolic activity of bovine spermatozoa [20].

Wheat germ is a concentrated source of several nutrients, including vitamins, minerals and unsaturated fatty acids (oleic and linoleic acids) [21].

Wheat germ is an excellent source of polyunsaturated fatty acids and vitamin E. These substances have been used for improving fertility and as antioxidant [22]. The antioxidant capacity of wheat germ is referred mainly to its vitamin E content as it is considered as a major chain- breaking antioxidant that plays a vital role in protecting spermatozoal membrane from oxidative damage, trapping and scavenging all three types of free radicals, namely superoxide, H_2O_2 and hydroxyl radicals [20], generated during conversion of lipid hydroperoxides in the peroxidative chain reaction [23,24]. Ejaculate volume and sperm concentration have been enhanced in vitamin E supplemented boars [25], and in semen extenders supplemented with vitamin E, resistance to lipid peroxidation have been ameliorated after semen storage [26]. Vitamin E supplementation improved post freezing sperm motility, mitochondrial membrane function and membrane integrity [27,28].

Materials and Subject/Methods

Buffalo bulls

Four buffalo bulls (aged 3.5 - 5.0 years) kept at the Abassia Buffalo Semen Freezing Center, Central Organization for Veterinary Services, Ministry of Agriculture, Egypt, were selected for semen collection. The buffalo bulls were maintained under uniform standard nutrition and managerial practices. They were in a good general health condition (600-800 kg body weight).

Preparation of different semen extenders

TRIS base extender

Tris-citric acid-fructose diluent (TCF) was prepared according to Foote., *et al* [29]. 10% whole egg yolk (TCFY) was added.

Preparation of wheat germ extract

3.75 gm wheat germ was added to 300 ml MeoH (70%) to extract both water soluble and alcoholic soluble ingredients. Stirring for seven days in the magnetic stirrer. Filtration and evaporation of the filtrate at 40ºC. The remaining stock solution (68 ml distilled water containing all the extract ingredients) [30] with little modification.

Wheat germ enriched extender

Five tubes were prepared. The first tube contains 0 Wheat germ extract in 4 ml tris extender and kept as a control. The other four tubes contain wheat germ extract as follows (250 µl stock/3.75 ml tris, 500 µl/3.5 ml, 1000 µl/3 ml and 2000 µl/2 ml) to reach a final volume 4 ml in each tube.

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 minutes at 37°C in the water bath before dilution.

Semen processing

Egg yolk was added to TCF extender at a concentration of 20% to constitute TCFY.

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 wheat germ extract to reach concentration of 60 million sperm/ml. Extended semen was cooled slowly (approximately for 2 hrs) to 5°C and equilibrated for 2 hrs. 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 minutes and were then plunged in liquid nitrogen.

Evaluation of semen quality parameters

The assessment was implemented post cooling and on chilled semen kept at 5°C daily. The parameters studied were subjective semen characteristics (motility, alive, abnormality, hyposmotic swelling test (HOST) and acrosome status) [31].

In vivo fertility rate (CR)

No. of cattle females (n = 290) were inseminated with the TW post-thawed semen and with the post-thawed semen extended in TCFY (control group). 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:

$$CR = \frac{\text{no.of conceived cattle}}{\text{total no.of inseminated cattle}} \text{ X } 100$$

Statistical analysis

Statistical analysis data were analyzed using the SPSS [32] computerized program v. 14.0 to calculate the analysis of variance (ANOVA) for the different parameters between control and additives replications. Significant difference between means was calculated using Duncan test at $P < 0.05$.

Results

Post-cooling results (Table 1) revealed improvement in sperm membrane integrity (HOST) in TW_1 followed by TW_2 if compared to the control (61.00 ± 1.00 and 48.33 ± 1.67 and 43.33 ± 1.67 respectively). Acrosome status was superior in TW_1, TW_2 and TW_3 relative to the

control (81.00 ± 1.00, 83.66 ± 1.86, 79.66 ± 3.17 and 66.50 ± 1.50 respectively). Sperm motility and livability were kept as the control. Sperm abnormalities were maintained as the control in TW_1 and TW_2 and deteriorated in TW_3 and TW_4.

	Motility	Alive	Abnormalities	HOST	Acrosome
TW_1	91.00 ± 1.00^{c}	84.00 ± 1.0^{ab}	$8.66 \pm .33^{ab}$	61.00 ± 1.00^{c}	81.00 ± 1.00^{b}
TW_2	85.33 ± 3.18^{bc}	88.00 ± 1.53^{b}	$7.00 \pm .58^{a}$	48.33 ± 1.67^{c}	83.66 ± 1.86^{b}
TW_3	81.66 ± 1.67^{ab}	83.00 ± 1.73^{ab}	16.00 ± 2.08^{c}	37.33 ± 2.70^{ac}	79.66 ± 3.17^{b}
TW_4	78.33 ± 1.67^{a}	81.33 ± 3.52^{a}	13.33 ± 2.84^{bc}	$40.66 \pm .67^{ab}$	67.66 ± 2.34^{a}
control	86.00 ± 1.00^{bc}	$89.33 \pm .66^{b}$	$8.33 \pm .33^{ab}$	43.33 ± 1.67^{b}	66.50 ± 1.50^{a}
Total	84.46 ± 1.34	85.13 ± 1.09	10.66 ± 1.09	46.13 ± 2.29	76.35 ± 2.10
p-value	.008	.072	.012	.000	.001

Table 1: *Effect of wheat germ extract on Post-cooled cattle bull semen quality (mean ± SE). Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at P < 0.05. Tw denotes Tris wheat germ.*

Post-thawing findings showed significant (P < .000) improvement in sperm motility in TW_1, TW_2, TW_3 if compared to TW_4 and the control (55.00 ± 2.88, 48.33 ± 1.66, 48.33 ± 1.66, 16.66 ± 3.33 and 36.66 ± 1.66 respectively). Alive sperm percent was significantly higher in TW_1, TW_2 and TW_3 if compared to TW_4 and the control (82.33 ± 1.45, 88.00 ± 1.53 83.00 ± 1.73, 60.00 ± 1.15 and 89.33 ± .66 respectively). Sperm abnormalities was significantly lower in TW_1, TW_2, TW_3 if compared to TW_4 (7.00 ± 1.15, 9.33 ± .66, 6.66 ± .88 and 10.67 ± .67 respectively). Sperm membrane integrity (HOST) were significantly higher in TW_1, TW_2 if compared to TW_3, TW_4 and the control (51.33 ± 1.33, 55.00 ± 1.73, 32.67 ± 1.45, 34.00 ± 2.31 and 25.00 ± 2.89 respectively).

	Motility	Alive	Abnormalities	HOST	Acrosome
TW_1	55.00 ± 2.88^{c}	82.33 ± 1.45^{c}	7.00 ± 1.15^{a}	51.33 ± 1.33^{c}	$51.63 \pm .86^{a}$
TW_2	48.33 ± 1.66^{c}	$82.00 \pm .57^{c}$	$9.33 \pm .66^{ab}$	55.00 ± 1.73^{c}	52.83 ± 1.42^{a}
TW_3	48.33 ± 1.66^{c}	$83.33 \pm .88^{c}$	$6.66 \pm .88^{a}$	32.67 ± 1.45^{b}	48.33 ± 1.67^{a}
TW_4	16.66 ± 3.33^{a}	60.00 ± 1.15^{b}	$10.67 \pm .67^{c}$	34.00 ± 2.31^{b}	51.10 ± 1.10^{a}
control	36.66 ± 1.66^{b}	54.33 ± 1.45^{a}	$8.00 \pm .58^{ab}$	25.00 ± 2.89^{a}	63.63 ± 2.70^{b}
Total	41.00 ± 3.72	63.60 ± 3.55	8.33 ± .50	39.60 ± 3.18	53.51 ± 1.55
p-value	.000	.000	.031	.000	.001

Table 2: *Effect of wheat germ extract on Post-thawed cattle bull semen quality (mean ± SE). Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at P < 0.05. Tw denotes TRIS wheat germ.*

Treatment	*In vivo* fertility rate (CR %)
TW_1	75.6%
TW_2	70.6%
TW_3	72 %
TW_4	33%
Control (TCFYG)	40.2%

Table 3: *Effect of Tris extender enriched with wheat germ extract on a field conception rate test in cattle bulls.*

Discussion

Semen cryopreservation results in a large degree of physical, chemical and mechanical injuries to sperm membrane [7] due to over accumulation of ROS causing oxidative damage [20,33] with subsequent decrease in sperm viability and motility [34,35]. The results of the current study revealed enhancement of the sperm characteristics post cooling and post-thawing with the first three concentrations of the wheat germ extract and deteriorated with the fourth concentration. Wheat is an excellent source of polyunsaturated fatty acids and vitamin E. The association of dietary Ω-3 fatty acids with vitamins E and C has improved semen quality in rabbits during semen storage [36]. In buffalo feeding of sunflower oil or sunflower seed rich in polyunsaturated fatty acids has resulted in improvement of the spermatozoa quality [37].

Vitamin E combined with 1% Nano-Se improved the post-thawing quality and oxidative variables of rooster semen [38]. The wheat germ constitutes only about 2% of the whole wheat grain and contains about 8 - 14% oil [39].

Wheat germ oil has the highest tocopherol content of all vegetable oils, up to about 2500 mg/kg [40], and also the highest content of α-tocopherol, which represents around60% of the total content. Also, wheat germ oil is of high nutritional value due to its high content of unsaturated fatty acids of which it contains about 80%, mostly linoleic and linolenic [22].

Wheat germ extract has strong antioxidant capacity due to its high contents of unsaturated fatty acids, vitamins and minerals. Unsaturated fatty acids and vitamins [41,42]; minerals [43] are strong antioxidant that improved the quality of preserved semen through elimination of the excess oxygen free radicals [44,45] as indicated by decreased malondialdehyde [46]. Wheat germ oil has been supplemented for improving fertility and as an antioxidant additive in natural food and health and cosmetic products [22].

Conception rate was the best in TW_1 followed by TW_2 and TW3and the lowest was in TW_4 and the control. These findings coincide with the enhanced sperm motility at these concentrations. These results are in agreement with those of Mahmoud., *et al*. [47] who showed that motility may be an applicant marker for semen quality, considering that significant correlations were found between motility and both sperm abnormalities and membrane integrity. Ramos and Wetzel's [48] reported that motility may be a relevant indicator for DNA integrity within sperm cells. Vale [49] recorded a pregnancy rate higher than 50% as a good result after AI with frozen thawed spermatozoa in buffalo Al Naib., *et al*. [50], classified bulls with pregnancy rate of about 50% to be considered of high fertility, and the sperm of high fertility bulls tends to be more effective in penetrating artificial mucus and to have an increased ability to fertilize oocyte *in vitro*.

Conclusion

It is concluded that, all concentrations of the wheat germ extract gave good post-cooling except the fourth concentration (TW_4). Post-thawing sperm parameters exhibited that sperm quality was superior in TW_1, TW_2, TW_3 and deteriorated with the fourth concentration (TW_4). Conception rate was the best in TW_1 followed by TW_2 and TW3and the lowest was in TW_4 and the control.

Acknowledgment

This work was supported by the National Research Centre [grant numbers: 10120801].

Conflict of Interest

The authors declare that they don't have any conflict of interest and all persons gave their informed consent prior to their inclusion in the study.

Bibliography

1. Parks JE and Lynch DV. "Lipid composition and thermotropic phase behavior of boar, bull, stallion, and rooster sperm membranes". *Cryobiology* 29.2 (1992): 255-266.

2. White IG. "Lipids and calcium uptake of sperm in relation to cold shock and preservation: a review". *Reproduction, Fertility and Development* 5.6 (1993): 639-658.

3. Flesch FM and Gadella BM. "Dynamics of the mammalian sperm plasma membrane in the process of fertilization". *Biochimica Biophysica Acta* 1469.3 (2000): 197-235.

4. Guthrie HD and Welch GR. "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* 84.8 (2006): 2089-2021.

5. Nair SJ., *et al.* "A 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* 96.1-2 (2006): 21-29.

6. Andrabi SMH. "Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa". *Reproduction Domestic Animals* 44.3 (2009): 552-569.

7. Watson PF. "The causes of reduced fertility with cryopreserved semen". *Animal Reproduction Science* 60-61 (2000): 481-492.

8. Kumar S., *et al.* "Cytomorphological characteristics of motile and static semen of buffalo bulls". *Buffalo Journal* 9.2 (1993): 117-127.

9. El-Azab A I., *et al.* "Effect of non-protein nitrogen in the ration on ram semen quality". *Small Ruminant Research* 27.1 (1998): 73-77.

10. Pramanik PS and Raina VS. "Refrigerator (4-70c) preservation of buffalo semen in various extenders". *Indian Journal of Dairy Science* 51 (1998): 375-379.

11. Akhter S., *et al.* "Effect of antibiotics in extender on bacterial and spermatozoal quality of cooled Buffalo (*Bubalus bubalis*) Bull Semen". *Reproduction in Domestic Animals* 43.3 (2007): 272-278.

12. Rasul Z., *et al.* "Changes in motion characteristics, plasma membrane integrity, and acrosome morphology during cryopreservation of buffalo spermatozoa". *Journal of Andrology* 22.2 (2001): 278-283.

13. Andrabi SMH., *et al.* "Duck egg yolk in extender improves the freez ability of buffalo bull spermatozoa". *Animal Reproduction Science* 104.2 (2008): 427-433.

14. Akhter S., *et al.* "*In vitro* evaluation of liquid-stored buffalo semen at 5 °C diluted in soya lecithin based extender (Bioxcell®), tris-citric egg yolk, skim milk and egg yolk-citrate extenders". *Reproduction in Domestic Animals* 46.1 (2011): 45-49.

15. Sansone G., *et al.* "Storage of buffalo (*Bubalus bubalis*) semen". *Animal Reproduction Science* 62.1 (2000): 55-76.

16. Bousseau S., *et al.* "Comparison of bacteriological qualities of various egg yolk sources and the *In vitro* and *In vivo* fertilizing potential of bovine semen frozen in egg yolk or lecithin based diluents". *Theriogenology* 50.5 (1998): 699-706.

17. Thibier M and Guerin B. "Hygienic aspects of storage and use of semen for artificial insemination". *Animal Reproduction Science* 62.1 (2000): 233-251.

18. El-Sherbieny MA. "Impact of replacing egg yolk with lecithin on sperm characteristics, bacterial count and fertilizing ability of cryopreserved buffalo semen". *Journal of Animal and Poultry Production* 5.6 (2014): 353-364.

19. Althouse GC. "Sanitary procedures for the production of extended semen". *Reproduction in Domestic Animals* 43.2 (2008): 374-378.

20. Câmara DR., *et al.* "Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen". *Small Ruminant Research* 100.1 (2011): 44-49.

21. Cohen, Allen Carson: Nutritional Components of Wheat Germ, Soy, Egg Yolk, Broccoli Florets and Beef Liver". 10 great health foods for eating well (2009).

22. Wang T and Johnson LA. "Reining high-free fatty acid wheat germ oil". *Journal of the American Oil Chemists' Society* 78 (2001): 71-76.

23. Aitken RJ and Clarkson JS. "Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques". *Journal of Andrololog*y 9.6 (1988): 367-376.

24. Therond P., *et al.* "α-Tocopherolin human spermatozoa and seminal plasma: relationships with motility, antioxidant enzymes and leukocytes". *Molecular Human Reproduction* 2.10 (1996): 739-744.

25. Brezezinska-Slebodzinska E., *et al.* "Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma". *Biological Trace Element Research* 47 (1995): 69-74.

26. Cerolini S., *et al.* "Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage". *Animal Reproduction Science* 58.1-2 (2000): 99-111.

27. Pena FJ., *et al.* "Antioxidant supplementation *In vitro* improves boar sperm motility and mitochondrial membrane potential aZer cryopreservation of different fractions of the ejaculate". *Animal Reproduction Science* 78 (2003): 85-98.

28. Pena FJ., *et al.* "Antioxidant supplementation of boar spermatozoa from different fractions of the ejaculate improves cryopreservation: changes in sperm membrane lipid architecture". *Zygote* 12.2 (2004): 117-124.

29. Foote RH., *et al.* "Motility and fertility of bull sperm in whole milk extender containing antioxidants". *Animal Reproduction Science* 71 (2002): 13-23.

30. Piras A., *et al.* "Extraction of oil from wheat germ by supercritical CO_2". *Molecules* 14.7 (2009): 2573-2581.

31. Salisbury GW., *et al.* "Semen evaluation: In "Physiology of Reproduction and Artificial Insemination of Cattle". 2nd edition. W.H. Freeman and Company, San Francisco, USA (1978): 400-427.

32. SPSS v.14.0 for Windows Evaluation Version Release (2005): 14.0.0.

33. Ortega Ferrusola C., *et al.* "Lipid peroxidation, assessed with BODIPY-C11, increases after cryopreservation of stallion spermatozoa, is stallion dependent and is related to apoptotic-like changes". *Reproduction* 138.1 (2009): 55-63.

34. Baumber J., *et al.* "The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation". *Journal of Andrology* 21.6 (2000): 895-902.

35. Agarwal A., *et al.* "Prevention of oxidative stress injury to sperm". *Journal of Andrology* 26.6 (2005): 654-660.

36. Castellini C., *et al.* "Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids". *Reproduction Nutrition Development* 43.1 (2003): 91-103.

37. Adeel M., *et al.* "Improvement of liquid and frozen thawed semen quality of Nili-Ravi buffalo bulls (*Bubalus bubalis*) through supplementation of fat". *Theriogenology* 71.8 (2009): 1220-1225.

38. Safa S., *et al.* "Effect of vitamin E and selenium nano particles on post-thaw variables and oxidative status of rooster semen". *Animal Reproduction Science* 174 (2016): 100-106.

39. Zacchi P., *et al.* "Extraction/ fractionation and deacidication of wheat germ oil using supercritical carbon dioxide". *Brazilian Journal of Chemical Engineering* 23.1 (2006): 105-110.

40. Shuler P. "Natural antioxidants exploited commercially". In: Hudson B.J.F. (edition): Food Antioxidants. Elsevier Applied Science, London: Chapter (1990).

41. Zhou C., *et al.* "Variation of oleanolic and ursolic acid in the flesh of persimmon fruit among different cultivars". *Molecules* 15.9 (2010): 6580-6587.

42. Karakasova L., *et al.* "Quality properties of solar dried persimmon (Diospyros kaki)". *Journal of Hygienic Engineering and Design* 4 (2013): 54-59.

43. Marqués AM., *et al.* "Mineral profile of kaki fruits (Diospyros kaki L.)". *Food Chemistry* 172 (2015): 291-297.

44. El-Sheshtawy RI., *et al.* "Effect of honey solution on semen preservability of local breeds of cattle bulls". *World Applied Science Journal* 32.10 (2014): 2076-2078.

45. El-Sheshtawy RI., *et al.* "The Effect of *Phoenix dactylifera* pollen grains tris-infusion on semen preservability of local bull breeds". *Global Veterinarian* 13.5 (2014): 728-732.

46. Zahmatkesh E., *et al.* "Protective effect of royal jelly on the sperm parameters and testosterone level and lipid peroxidation in adult mice treated with oxymetholone". *Avicenna Journal of Phytomedicine* 4.1 (2014): 43-52.

47. Mahmoud KGM., *et al.* "Sperm characteristics in cryopreserved buffalo bull semen and field fertility". *Iranian Journal of Applied Animal Science* 3.4 (2013): 777-783.

48. Ramos L and Wetzels AM. "Low rates of DNA fragmentation in selected motile human spermatozoa assessed by the tunel assay". *Human Reproduction* 16.8 (2001): 1703-1707.

49. Vale WG. "Sperm cryopreservation". *Bubalus Bubalis* 1 (1997): 129-140.

50. Al Naib A., *et al.* "*In vitro* assessment of sperm from bulls of high and low fertility". *Theriogenology* 76.1 (2011): 161-167.

Volume 5 Issue 10 Octosber 2020