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Original Article

Assessment of Buffalo Semen Preservability Using Tris Extender Enriched With Turmeric Extract

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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. Running title: Assessment of Tris Turmeric Extender on buffalo semen .

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Abstract

Background and Objective: The freeze-thaw process results in structural and functional damages caused by over-accumulation of reactive oxygen species (ROS). Addition of exogenous antioxidants to semen extender is of a great importance to overcome the oxidative damage during the freezing process antioxidants to semen extender is of a great importance to overcome the oxidative damage during the freezing process. The objective was to evaluate the effect of tris citric acid fructose egg yolk (TCFY) extender supplemented with turmeric extract .

Materials and Methods:Five tubes (each contains 5ml TCFY).The first tube contains no turmeric extract and kept as a control. The other four tubes contain turmeric extract as follows (100µl /5ml, 200 µl /5ml, 300 µl /5ml and 400 µl /5ml, v/v). Pooled bull semen were extended with Tris extender (Tris with zero turmeric extract was kept as a control) . Turmeric extract was added to the tris extender at concentrations (100µl /5ml TT_1, 200 µl /5ml TT_2, 300 µl /5ml TT_3 and 400 µl /5ml, TT_4 v/v).. The semen samples was added and final sperm concentration 60×10^6/ml was attained. Extended semen were subjected to semen freezing protocol. Semen assessments including motility, alive%, abnormality%, intact sperm membrane (hypo-osmotic swelling test), and acrosome status were carried out for both cooled and frozen semen. Conception rate was carried out with the post-thawed semen.

Results: The post-cooling semen characteristics revealed improved sperm motility inTT_1 and TT_2, Sperm membrane integrity (HOST) significantly (P<0.016) ameliorated inTT_3 and acrosome integrity significantly ((P<0.037) enhanced in TT_1 as compared to the control. The post-thawing results exhibited significant (P<0.000) improvement in sperm motility in TT_1 and TT_2 and significant (P<0.004) amelioration in Sperm membrane integrity (HOST) inTT_1, $TT_3$and TT_4 if compared to the control. The conception rate was the best in concentrations TT_1 and TT_2 if compared to the control and other concentrations.

Conclusion: It is concluded that, in cooled and post-thawed semen, the superior semen quality and Conception rate was attained in TT_1 and TT_2.

Keywords: Buffalo; Turmeric Extract; semen; Tris; cryopreservation.

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Introduction

Improvement of semen cryopreservation of the buffalo bulls is great objective, this could be achieved through supplementation of the extended semen with an 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 [1] 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 [2]. Curcumin is a polyphenolic insoluble in water that scavenges free radicals [3] 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 [4]. Administration of curcumin to male rodents improved testicular function and fertility [5,6].

Curcumin is the principal of curcuminoid of turmeric (curcuma longa), a member of ginger family. Curcuminoids are natural phenols responsible for turmeric is yellow colour [7] .Turmeric extract contain curcumin with other curcuminoids and essential oils which were found to be bioactive [8].

MATERIALS AND METHODS

1.1. Preparation of different semen extenders:

TRIS base extender: Tris-citric acid-fructose diluent (TCF) was prepared according to Foote et al. [9] . 20% whole egg yolk (TCFY) was added.

b- **Preparation of turmeric extract:** 4 gm turmeric powder+60mlethanol in a test tube.

4gm turmeric powder+60mldistilled water in another tube. Using stirrer for mixing in each tube, filtration. The filterate is left at 40c for 24hrs till evaporation. The residues in both tubes were mixed together and dissolved in 2ml tris and kept as a stock solution.

Turmeric enriched extender [TEE]: Five tubes (each contain 5ml TCFY).The first tube contains 0 turmeric extract and kept as a control. The other four tubes contain turmeric extract as follows (100µl /5ml, 200 µl /5ml, 300 µl /5ml and 400 µl /5ml, v/v).

1.2. 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 minute at 37°C in the water bath before dilution.

1.3. 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 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 [10].

1.4. 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 minute. The parameters studied were subjective semen characteristics (motility, alive, abnormality, hypoosmotic swelling test (HOST) and acrosome status [11].

1.5. Statistical analysis:

Statistical analysis data were analyzed using the SPSS [12] 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 $P<0.05$.

1.6. In vivo fertility rate (CR):

No. of buffalo females (n=270) were inseminated with the TT 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:

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

RESULTS

The post cooling semen characteristics revealed improved sperm motility inTT_1 and TT_2, Sperm membrane integrity (HOST) significantly (P<0.016) ameliorated inTT_3 and acrosome integrity significantly ((P<0.037) enhanced in TT_1 as compared to the control . The post thawing results exhibited significant (P<0.000) improvement in sperm motility in TT_1 and TT_2 and significant (P<0.004) amelioration in Sperm membrane integrity (HOST) inTT_1 ,$TT_3$and TT_4 if compared to the control . Conception rate was the best in concentrations TT_1 and TT_2 if compared to the control and other concentrations.

Table 1. Effect of Tris extender enriched with Turmeric extract on the cooled extended buffalo bull semen (Mean±SE)

Diluent	Motility	Alive	Abnormalities	Host	Acrosome
TT_1	93.33±1.66^{a}	91.62±.90^{a}	10.00±1.15^{a}	52.29±3.43^{a}	91.66±1.20^{b}
TT_2	93.33±1.66^{a}	89.33±1.20^{a}	6.33±1.76^{a}	55.72±.41^{a}	83.33±1.66^{a}
TT_3	88.33±1.66^{a}	91.00±.57^{a}	7.00±.57^{a}	69.08±3.69^{b}	88.33±1.66^{ab}
TT_4	88.33±1.66^{a}	92.00±3.51^{a}	10.00±1.52^{a}	60.54±3.56^{ab}	88.33±1.66^{ab}
Control	88.33±1.66^{a}	85.00±2.88^{a}	7.33±.88^{a}	49.95±4.11^{a}	88.00±2.00^{ab}
Total	90.33±.90	89.79±2.88	8.13±.63	57.52±2.20	87.92±.97
p-value	.092	.212	.183	.016	.037

Means bearing different superscripts between different extenders and differ at 5% and 1% levels of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG); TT_1(TrisT_1); TT_2(TrisT_2); TT_3(TrisT_3).

Table 2. Effect of Tris extender enriched with Turmeric extract on the post- thawed extended buffalo bull Semen (Mean±SE).

Diluent	Motility	Alive	Abnormalities	Host	Acrosome
TT_1	61.66±1.66^{b}	85.00±1.73^{a}	7.33±.66^{a}	77.93±2.56^{b}	85.67±2.90^{b}
TT_2	60.00±.00^{b}	84.66±2.33^{a}	6.66±1.20^{a}	63.56±6.18^{a}	83.33±3.48^{ab}
TT_3	41.66±1.66^{a}	85.33±3.66^{a}	7.66±2.02^{a}	74.18±2.26^{b}	76.00±1.73^{a}
TT_4	41.66±1.66^{a}	88.66±2.33^{a}	10.00±1.73^{a}	75.30±.30^{b}	83.66±2.33^{ab}
Control	43.33±1.66^{a}	85.33±2.02^{a}	10.00±1.15^{a}	57.33±.33^{a}	81.66±1.66^{ab}
Total	49.66±2.51	85.80±1.02	8.33±.65	69.66±2.42	82.06±1.29

Diluent	Motility	Alive	Abnormalities	Host	Acrosome
TT_1	61.66±1.66[b]	85.00±1.73[a]	7.33±.66[a]	77.93±2.56[b]	85.67±2.90[b]
TT_2	60.00±.00[b]	84.66±2.33[a]	6.66±1.20[a]	63.56±6.18[a]	83.33±3.48[ab]
TT_3	41.66±1.66[a]	85.33±3.66[a]	7.66±2.02[a]	74.18±2.26[b]	76.00±1.73[a]
TT_4	41.66±1.66[a]	88.66±2.33[a]	10.00±1.73[a]	75.30±.30[b]	83.66±2.33[ab]
Control	43.33±1.66[a]	85.33±2.02[a]	10.00±1.15[a]	57.33±.33[a]	81.66±1.66[ab]
Total	49.66±2.51	85.80±1.02	8.33±.65	69.66±2.42	82.06±1.29
p-value	.000	.791	.376	.004	.152

Means bearing different superscripts between different extenders and differ at 5% and 1% levels of probability. Control Tris-citrate-fructose-egg yolk-glycerol (TCFYG); TT_1 ($TrisT_1$); TT_2 ($TrisT_2$); TT_3 ($TrisT_3$).

Table 3: Effect of Tris extender enriched with Turmeric extract on a field conception rate test in buffalo bulls.

Treatment	In vivo fertility rate (CR %)
TT_1	77.6%
TT_2	75.6%
TT_3	40 %
TT_4	45%
control(TCFYG)	40.2%

DISCUSSION

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 [13] Semen freezing causes damage to spermatozoa leading to reduction in semen quality [14], 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 [15] So, the natural additive to the extender ameliorates the antioxidant effect and consequently improving the fertilizing capacity of frozen spermatozoa [16]. The post cooling , post thawing semen characteristics and conception rate in our study were improved upon using Tris enriched with Turmeric as a cryoprotectant in the bull semen extender. The best conception rate in TT_1 and TT_2 coincide with the best sperm motility at these concentrations Curcumin is the major extract of turmeric, it is a lipophilic polyphenol insoluble in water and scavenge free radicals, significantly inhibit the generation of (ROS) [1]. Curcumin significantly increase the sperm content of GSH, thus improving the antioxidant capacity of the semen extender [4] .Curcumin shows antioxidant activity through binding with egg and soyphosphatidylcholine which in turn binds divalent metal ions and has antibacterial and antiviral effects [17].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 [18].

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 [19].

Conclusion

It is concluded that , in cooled and post thawed semen , the superior semen quality was attained in TT_1 and TT_2 .

Ethics approval and consent to participate

The present study design was approved ethically by the Medical Research Ethics Committee at the National Research Centre, Dokki, Cairo, Egypt, with a registration number 19/043.

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Conflict of interest: I am a single author ,so there is not any conflict of interest.

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