



Effect of Royal Jelly Tris-enriched Extender on Buffalo Semen Cryopreservation

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THE objective was to assess the consequence of tris-extender enriched with different concentrations of Royal jelly on buffalo bull semen preservability. Pooled semen was diluted with tris (control, 0% Royal jelly) and variable concentrations of Royal jelly in tris (TR) (0.05, 0.1, 0.2, 0.3 and 0.4%). Diluted semen was exposed to semen freezing procedures. Semen assessment was carried out for both cooled and frozen semen. In cooled semen, sperm parameters were maintained as the control. The second concentration gave the best sperm membrane integrity (HOST) percent, while the lowest was at the 0.4% concentration. Acrosome integrity was the highest in the 0.1% concentration ..

Sperm motility of post-thawed frozen semen exhibited that, the superior was given with the 0.05, 0.1 and the 0.2% concentrations of royal jelly while ,the lowest was at the last two concentrations (0.3 and 0.4%). Alive sperm percent was maintained in all the concentrations as the control .The HOST percent was significantly higher in the five concentrations and the best was given with the 0.05% concentration . It is concluded that ,in cooled semen the 0.1% concentration gave the best sperm quality, while the lowest was at the 0.4% concentration.. Sperm parameters of post-thawed frozen semen exhibited that, the superior was given with the 0.05%, 0.1% and the 0.2% concentrations of royal jelly while ,the lowest was at the last two concentrations (0.3 and 0.4%). . Conception rate was the best in Tris royal jelly (TR₁, TR₂ and TR₃) and decreased at the last two concentrations .

Keywords: Buffalo, Semen, Cryopreservation, Royal Jelly.

Introduction

The freeze-thawing procedure results in morphological and functional deterioration produced by the excessive increase of reactive oxygen species (ROS) [1]. 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 [2, 3]. Cryopreservation process decreases the antioxidant seminal capacity. Therefore, adding of antioxidants to semen diluents is very essential to minimize the cryodamage during the freezing protocol [4].

Royal Jelly (bee's milk) is produced by the hypo-pharyngeal and mandibular glands of the worker honey bees and this is considered the primary food of the honey bee queen and honey bee larvae during the first three days of its life [5]. Royal jelly is composed of proteins, lipids, sugars, vitamins and essential amino acids [6,7]. Royal jelly has a beneficial biological activity in different cells and tissues of laboratory animals [8]. Royal jelly has antibacterial effect [9]. The objective of this study was to elucidate the protective effect of royal jelly addition in semen extender on semen quality post-cooling and post-freezing and on conception rate.

Materials and Methods

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 held for 10 minute at 37°C in the water bath before dilution.

Semen processing.

The basic extender was Tris-citric acid-fructose (TCF) that was prepared according to Foote1970[10]. 20% whole egg yolk (TCFY) was added. RJ was added to the tris extender at concentrations (0, 0.05, 0.1, 0.2, 0.3 and 0.4%). The semen samples was added and final sperm concentration $60 \times 10^6/\text{mL}$ was attained. Extended semen samples with 0 RJ was used as control and other aliquots of pooled semen samples containing the different concentrations of RJ were kept as experimental. 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 [11].

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) [12].

In vivo fertility rate (CR).

No. of buffalo females (n=310) were inseminated with the TR 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 buffalo cows were used via the cooperation in Beni-Suef Governorate. CR was computed according to the equation:

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

Statistical analysis

Data were analyzed using the SPSS [13] 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

In cooled semen, sperm motility and alive percent in the five concentrations of royal jelly were kept as the control. Sperm abnormalities were significantly reduced in the five concentrations if compared to the control. The second concentration gave the best HOST percent, while the lowest was at the fifth concentration if compared to the control. Acrosome integrity was the highest in the second concentration as compared to the control (Table 1).

Sperm motility of frozen semen exhibited that, the superior was given with the first, second and the third concentrations of royal jelly while, the lowest was at the last two concentrations. Alive sperm percent was maintained in all the concentrations as the control. The HOST percent was significantly higher in the five concentrations if compared to the control and the best were given with the first concentration. Acrosome integrity was not significantly higher in the first concentration as compared to the control and other concentrations (Table 2). Conception rate was the best in TR1, TR2 and TR3 and decreased at the last two concentrations (Table 3).

Discussion

Freezing procedures have deleterious changes on spermatozoa due to temperature changes, cold shock and ice crystals development [14,15,16,17]. These effects exert reduction in motility, plasma membrane and acrosome integrities and fertilizing ability of spermatozoa [15,18,19].

Reactive oxygen species (ROS) are produced as a consequence of unsaturated fatty acids peroxidation of the sperm membrane throughout the freeze-thawing procedures leading to its damage with subsequent reduction in motility and fertilizing ability. Antioxidants are used as a supplementary to the semen extenders to minimize fatty acids peroxidation and to reduce the hazardous effect in semen quality

TABLE 1. Effect of Royal jelly on Post-cooling buffalo semen quality (mean \pm SE).

	Motility	Alive	Abnormalities	HOST	Acrosome
TR ₁	88.33 \pm 1.67	89.00 \pm 2.08	14.33 \pm 1.20 ^b	69.69 \pm 0.7 ^{bc}	86.33 \pm 1.33 ^b
TR ₂	93.33 \pm 1.67	87.00 \pm 2.09	7.00 \pm 0.58 ^a	82.00 \pm 6.18 ^d	91.33 \pm 1.33 ^c
TR ₃	88.33 \pm 1.67	89.00 \pm 0.58	7.00 \pm 0.58 ^a	60.41 \pm 3.38 ^{ab}	86.00 \pm 1.00 ^b
TR ₄	93.33 \pm 1.67	91.33 \pm 1.33	8.00 \pm 0.58 ^a	79.14 \pm 4.64 ^{cd}	83.00 \pm 1.53 ^{ab}
TR ₅	93.33 \pm 1.67	91.67 \pm 1.67	9.67 \pm 0.33 ^a	56.06 \pm 3.11 ^a	80.67 \pm 0.67 ^a
Control	88.33 \pm 1.67	85.00 \pm 0.58	18.33 \pm 1.67 ^c	80.70 \pm 0.74 ^{cd}	80.67 \pm 0.67 ^a
Total	90.83 \pm 0.83	88.83 \pm 0.76	10.72 \pm 1.07	71.33 \pm 2.76	84.66 \pm 0.98
p-value	0.074	0.067	0.000	0.001	0.000

Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at P<0.05. R denotes Royal jelly.

TABLE 2. Effect of Royal jelly on Post-thawing buffalo semen quality (mean \pm SE).

	Motility	Alive	Abnormalities	HOST	Acrosome
TR ₁	63.33 \pm 1.66 ^b	86.33 \pm 1.33 ^a	10.33 \pm 0.33 ^b	83.58 \pm 4.77 ^b	91.66 \pm 1.66 ^b
TR ₂	63.33 \pm 1.66 ^b	82.66 \pm 2.66 ^a	10.33 \pm 0.33 ^b	75.08 \pm 0.34 ^b	86.33 \pm 1.33 ^{ab}
TR ₃	63.33 \pm 1.66 ^b	86.33 \pm 1.33 ^a	9.33 \pm 0.33 ^b	74.47 \pm 1.20 ^b	84.33 \pm 2.33 ^a
TR ₄	48.33 \pm 1.66 ^a	82.00 \pm 0.00 ^a	6.66 \pm 0.33 ^a	79.57 \pm 0.25 ^b	83.66 \pm 1.85 ^a
TR ₅	48.33 \pm 1.66 ^a	86.33 \pm 1.33 ^a	13.66 \pm 0.88 ^c	73.55 \pm 4.91 ^b	89.33 \pm 2.33 ^{ab}
Control	43.33 \pm 1.66 ^a	86.66 \pm 3.33 ^a	6.66 \pm 0.33 ^a	48.66 \pm 7.51 ^a	87.50 \pm 1.66 ^{ab}
Total	55.00 \pm 0.00	85.05 \pm 0.05	9.50 \pm 0.60	72.48 \pm 0.48	87.11 \pm 0.12
p-value	0.000	0.468	0.000	0.001	0.086

Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at P<0.05. R denotes Royal jelly.

TABLE 3. Effect of Tris Royal Jelly enriched extender on a field conception rate test in buffalo bulls.

Treatment	In vivo fertility rate (CR, %)
TR ₁	66.6%
TR ₂	65.6%
TR ₃	65 %
TR ₄	48%
TR ₅	45%
Control(TCFYG)	40.2%

parameters [20,18]. Royal jelly (RJ) is secreted from the mandibular and hypopharyngeal glands of young worker bees. It is white-yellowish in colour, sweet material. This material is a 3-day nourishment of the of worker bees larvae and also a larval and adult nutrient supply of the queens . Royal jelly (RJ) contains about 50%–70% water, 7%–18% carbohydrate, 9%–18% protein, 3–8% fatty acid and lipid, 1.5% mineral and low percent of vitamins and polyphenols [20,21].

The essential amino acids content of royal jelly exerts an antioxidant effect through elimination of the excess oxygen free radicals [22,23]. Royal jelly improved male fertility in laboratory animals [24,9,25] and on sperm quality during cooling [26]. Our results revealed that the first , second and the third concentrations of royal jelly (0.05, 0.1,0.2) gave the superior semen quality post cooling and post freezing. These findings agreed with Shahzad et al. [21] who recorded improved buffalo sperm motility upon using Tris–extender enriched with 0.05, 0.1, 0.2 and 0.3% than 0.4% supplemented royal jelly and control groups. They confirmed their results by implementing IVF and in vivo insemination where the cleavage and pregnancy rates were improved with 0.1 royal jelly concentrations. They added that, sperm livability, sperm membrane and acrosome integrities were considerably enhanced in 0.1 royal jelly supplemented group as compared to other groups contradicting with our results indicating that the first concentration (0.05) was the best in HOST and acrosome percent. The amino acids content in royal jelly enhances the sperm motility, acrosome reaction and consequently the fertilizing potential [27]. The short-chain fatty acids in royal jelly improve the sperm motility [28,29]. Royal jelly ameliorated the fatty acid peroxidation as indicated by the lowered levels of MDA (9, 25). Royal jelly has antioxidant effect on goat and fish semen freezing [20,30]. Royal jelly is one of the important antioxidant additives, since it has an excellent protective functions exerted from its amino acid content [20,21].

Motility is one of the essential semen quality parameter for oocyte fertilization [31,19]. In the present study, Sperm motility of frozen semen exhibited that, the superior was given with the first , second and the third concentrations of royal jelly while ,the lowest was at the last two concentrations.

These findings are in a good agreement with the findings of Alcay et al. [2019][30] who found that RJ supplementation caused an obvious increase on motility .However, higher concentrations of RJ revealed a slow decline in sperm motility.

The functional status of spermatozoal membrane that is essential for the sperm metabolic processes acting a fundamental part for sperm oocyte fusion . So, sperm membrane integrity is essential for oocyte fertilizing capacity of sperm [32]. Nevertheless, sperm membrane integrity and permeability are deteriorated from cold shock and fatty acids peroxidation. Cold shock result in lipid phase transition of the sperm membrane with loss of its discriminating permeability [33,34]. The protection from cold shock is achieved by improving the sperm membrane fluidity [35]. HOST is the specific test for indicating the delicate alterations of the spermatozoal membrane integrity [36]. In the current study, the plasma membrane integrity parameters were higher than the control at post thaw. These results are in agreement with Alcay et al. [15,30].

Acrosomal damage is one of the adverse effects of cryopreservation [15,19,16]. Acrosomal integrity is linked to the fertilizing potential of the frozen spermatozoa due to its responsibility for zona pellucida fusion. In this study, Acrosome integrity was the highest in the first concentration as compared to the control and other concentrations . Our results agreed with Alcay et al. (2019) [30] . In this study, the superior in vivo sperm fertility expressed as conception rate (CR) of the post-thawed frozen semen used in artificial insemination were given with the first , second and the third concentrations of royal jelly and decreased at the last two concentrations . These findings coincide with the enhanced sperm motility at these concentrations . These results are in agreement with those of Mahmoud et al. [37] who showed that motility may be an applicant indicator for semen quality, based on that marked correlations were observed between motility and each of the sperm morphological abnormalities and membrane integrity. Ramos and Wetzel's [38] recorded that motility may be a strong indicator for DNA integrity of the sperm cells .Vale [39] recorded a conception rate higher than 50% as a good outcome post AI with frozen- thawed spermatozoa in buffalo Al Naib et al. [40] , categorized bulls with pregnancy

rate of about 50% to be highly fertile, and the sperm of highly fertile bulls tends to be more efficient in penetrating artificial mucus and to have an increased capability to fertilize oocyte in vitro. So, in the present study all concentrations of Royal Jelly are considered ameliorating for sperm fertility (conception rate) and the superior was at the first three concentrations. It can be concluded that, Sperm parameters of post-thawed frozen semen were superior with the first (TR₁), second (TR₂) and the third (TR₃) concentrations of royal jelly while, the lowest was at the last two concentrations and conception rate was the best in TR₁, TR₂ and TR₃ and decreased at the last two concentrations.

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Conflict of interest

I am the single author, so there isn't any conflict of interest.

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Ethical consideration

The present study is ethically approved by the medical research ethics committee of the National Research Centre. The ethics number is 19104

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تأثير التريس المدعم بغذاء ملكات النحل علي كفاءة الحفظ بالتجميد للسائل المنوي للجاموس

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الهدف من هذا البحث هو تقييم كفاءة حفظ السائل المنوي المخفف بالتريس المدعم بغذاء ملكات النحل . تم تخفيف السائل المنوي المجمع بمخفف التريس فقط ككنترول بالاضافة الي التريس المدعم بغذاء ملكات النحل بتركيزات مختلفة (٠,٠٥ ، ٠,١ ، ٠,٢ ، ٠,٣ ، ٠,٤) . تم عمل تبريد وتجميد للسائل المنوي المخفف. أظهرت النتائج أن خصائص السائل المنوي المبرد تقريبا مثل الكنترول . اما السائل المنوي المجدد تحسنت صفاته مقارنة بالكنترول . و الخلاصة أن أفضل النتائج بعد التجميد والاذابة كانت في التركيزات (٠,٠٥ ، ٠,١ ، ٠,٢) بينما كانت الاقل في التركيزين (٠,٣ ، ٠,٤) .