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EFFECTS OF ROYAL JELLY ON POST THAW SEMEN QUALITY PARAMETERS OF BEETAL BUCK

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The aim of this study was to compare the effects of using different levels of royal jelly in semen extender on vital characteristics parameters of goat sperm after freezing. Semen was collected from three adult bucks twice a week and samples pooled together. Sperm samples (seven repeats) containing royal jelly in five levels (0,0.5, 1, 1.5 and 2%) had been frozen. After freezing semen samples were thawed and examined in terms of motility, survival and integrity of the plasma membrane, normal acrosome and DNA integrity . Average total motility, livability, plasma membrane integrity and DNA % at 1% royal jelly were higher than other treatment and control groups ($P<0.05$). Therefore, the use of royal jelly in goat semen diluent can improve some sperm parameters after freezing process.

Keywords: Royal Jelly, Beetal, Semen, Extender, motility

In goat genetic improvement artificial insemination is the most important tool and a great development in goat reproduction (Lopez-Sebastián, Coloma et al., 2014). because it has an important role in control of reproduction i.e. kidding at a precise time of the year (Leboeuf, Restall et al., 2003) Cryopreservation steps such as dilution, cooling process of two hours, equilibrium time and freezing weaken the normally existing antioxidant capacity of semen. In this context, to improve the semen quality and to combat stress produced by oxidation different exogenous antioxidant has been added in semen extender(Bansal and Bilaspuri, 2010) However, the biggest problem with cryopreserved spermatozoa is that free-thaw process deteriorate the sperm parameters i.e. reduction in sperm motility percentage, viability of the spermatozoa due to damage to membrane integrity and ultrastructure, decrease fertility(Dorado, Munoz-Serrano et al., 2010).

Royal jelly (RJ) secreted from the mandibular and hypopharangeal gland of the worker bee at six to twelfth week of their life consists proteins, sugars, lipids, free amino acids and vitamins. The overall composition of royal jelly is 67% water, 12.5% crude protein (including small amounts of many different amino acids), and 11% simple sugars (monosaccharide), also including 5% fatty acids. It contains vital vitamins which have antioxidant potency like vitamin E other vitamins are A, B5 ,C and essential amino acid especially lysine, cysteine and arginine(Howe, Dimick et al., 1985). It has been documented that scavenging ability against hydroxyl radical and high antioxidant potency is due to amino acid present in royal jelly(Karadeniz, Simsek et al., 2011; Robak and Marcinkiewicz, 1994).

MATERIALS AND METHODS

Animal and collection

Three mature regular semen donors Beetle bucks managed under uniform management, environmental and feeding conditions stationed at Al-Haiwan Sires, Sahiwal, Punjab Pakistan was used for this experiment.

Semen was collected twice a week using artificial vagina at 42ºC from each buck. Each ejaculate was transferred to laboratory immediately, placed in water bath at 37ºC. Semen was evaluated for volume, color, mass motility, sperm concentration and progressive motility. Ejaculates containing >70% motility and concentration $2x10^9$ was pooled and considered for preservation.

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Processing

Basic extender was used Tris-Citric Acid (TCA) as a buffer. It was prepared by following composition adding 3.07g TRIS, 1.56g Citric Acid, 0.2g fructose (Naijian et al., 2013), 10,000 IU benzyl penicillin in distill water and heated for 45 minute at 60 degree then cooled and mixed it with 5% glycerol and 15 % egg yolk (Naijian, Kohram et al., 2013) and make volume up to 100 ml .Then extender was divided into five aliquots for Royal Jelly treatment named as RJ0, RJ0.5, RJ1, RJ1.5 and RJ2(Shahzad, Mehmood et al., 2016) containing equal volume. Now concentrations of royal jelly was added in this extender and mixing was done with magnetic stitiror. Semen is filled in straws and kept for cooling in cooling box for 4 hours. After 4 hours semen was kept on cooling vapor for 7 minute and then deep freezing was done. After 24 h of storage in liquid nitrogen, three semen straws from each treatment group in each replicate were thawed at 37◦C for 30 s and analyzed for the following parameters.

Motility

The sperm motility was evaluated subjectively by putting one drop of semen (5 µl) from every semen sample on pre-warmed microscopic glass slide. It was enclosed by a coverslip using a phase contrast microscope at warmed stage. Assessment of motility was done in five widely spaced microscopic areas. Progressive motility was considered of those sperm moving rectilinear forward motion. The mean of this motility was recorded as the final motility score.

Assessment of viability and acrosomel status:

A combination of FITC-PNA (it colors damaged acrosome) and PI (it colors damaged plasma membrane) was used to study simultaneously live percentage and normal acrosomel status of spermatozoa. A final concentration of 10 and 25 µg/ml of FITC-PNA and PI, respectively was used to stain sperm suspensions. A 10 µl aliquot of the double-stained sperm suspension will be used after incubation for 30 min at 37 °C and will be observed under a fluorescence micro-scope (×100) at room temperature placing onto a microscopic slide, covered with a cover slip .For each slide two hundred sperm cells will be recorded. Four populations of sperms will be seen 1) Sperms with damaged acrosome will get green color. 2) Sperms with damaged plasma membrane will get red color. 3) Sperms with damaged acrosome and plasma membrane will get both colors (Green acrosome and red plasma membrane) 4) sperms with intact acrosome and plasma membrane will not get any stain(Sa-ardrit, Thongtip et al., 2012).

Plasma Membrane Integrity

PMI was assessed using Hypo-osmotic Swelling test. For HOS 10 µl of frozen-thawed semen sample with 100µl of HOS solution was mixed and incubated at 37oC for 45 min in hot air oven. After incubation one drop of sample was taken on glass slide, covered with coverslip and observed under phase contrast microscope. Two hundred spermatozoa in each sample will be assessed. Sperm having swollen or coiled tails of varying degrees was recorded to have an intact plasma membrane.

DNA integrity

This protocol was allowed us to differentiate between spermatozoa having intact DNA or denatured DNA. DNA integrity of spermatozoa was checked by using the acridine orange technique. A small drop of semen sample was taken on a glass slide and sample was allowed to air dried. After Air-drying these smear made slides was fixed in Carney's solution (three parts of methanol and one part glacial acetic acid) for period of two hours and finally slides was made to air dry. After drying of slides, these were rinsed with tempone solution for at least 5 minutes and were air dried. Finally these slides were stained with acridine orange stain for three minutes. These stained slides were examined immediately under a fluorescence microscope. Sperm cell having intact or normal DNA showed green fluorescence microscope, while sperm cells with damaged DNA or with abnormalities was showed varying fluorescence (from yellow green to red). Minimum hundred spermatozoa per smear were counted under the fluorescence microscope for DNA abnormalities.

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Statistical Analysis

Each treatment was replicated 5 times. For each replicate, thawing of three sperm straws was done at 37^0C for evaluation of sperm quality parameters. The repeated measures analysis of variance (ANOVA) under completely randomized design was performed for different concentration of RJ. Significance differences were compared by using Duncan Multiple Range (DMR) test. Results were expressed as mean ±SEM.

RESULTS

The effect of RJ supplementation in extender on progressive motility, viability, sperm plasma membrane, normal acrosome and chromatin integrity has been presented. The post-thaw results showed that 1% RJ supplemented significantly improved ($P < 0.05$) progressive motility compared to 0 and other RJ treated group. Sperm viability was significantly higher in 1%RJ group than other treatments groups. Higher percentage of sperm with intact membrane was observed in 1%RJ compared to control and other treatment groups. In addition, the sperm acrosome integrity was greater ($P < 0.05$) in 0.1% RJ than other treatment groups(0,0.5, 1.5 and 2%).The sperm DNA integrity was higher siginficantly ($P > 0.05$) in 1 % supplemented group..

DISCUSSION

The result of this study revealed that percentage motility of spermatozoa was significantly higher ($P>0.05$) at 1% concentration of royal jelly. The results of this study were similar to results of a study done by Qaiser shahzad. He used different concentration (0.05, 0.1, 0.2, 0.3 and 0.4 %) of royal jelly in semen extender and check the semen parameter after thawing, resulting significant increase of sperm motility at 0.1 % of royal jelly concentration(Shahzad, Mehmood et al., 2016). The results of this study were also similar with (moradi). He used different concentration(0, 0.5, 1, 1.5, and 2%) of royal jelly in extended semen of ram. He checked the sperm parameter at liquid storage. There was significant increase in sperm motility at 1% and 1.5%.

Livability% of sperm was checked by fitc PNA/Pi staining method. Post thaw live % of spermatozoa in semen supplemented with 1 % Royal Jelly showed significantly ($P<0.05$) highest value (53.60±0.80). Study on buffalo semen after post thaw by qaiser shahzad revealed increase in line % was up to 0.1 % and after this concentration live percentage was decreaed(Shahzad, Mehmood et al., 2016). In another study done by abduulah et al in semen of buffalo bull, live % of spermatozoa was increased at 0.5 % concentration of royal jelly(Abd–Allah, 2010).

Post thaw PMI of spermatozoa in semen added with 1 % Royal Jelly showed significantly ($P<0.05$) highest value (53.61±0.58).In recent study royal jelly shown a higher sperm plasma membrane integrity with 0.1 % concentration of post thaw quality of bull semen(Shahzad, Mehmood et al., 2016). In case of ram semen Plasma membrane integrity was also increased at 0.5 % royal jelly concentration(Moradi, Malekinejad et al., 2013).

Post thaw NAR % of spermatozoa in semen supplemented with 1 % Royal Jelly showed significantly ($P<0.05$) highest value (64.67±1.04) as compared to control (55.12±0.88) and other treatment groups .Same kind of study done for buffalo bull with various concentration of royal jelly. Post thaw NAR % was increased at 0.1 concentration of royal jelly which was 75.0 ± 6.4(Shahzad, Mehmood et al., 2016) .

DNA integrity of spermatozoa was significantly increased at 1% concentration of royal jelly. DNA integrity percentage at 1% was increased.

CONCLUSION

In conclusion, the structural and functional parameters of sperm during freezing-thawing can be augmented by supplementing the RJ in freezing extender and a doseof 0.1% RJ provide the best post-thaw sperm quality.

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