

## Research Article

# Edible Birds' Nest (EBN) Hydrolysate for Bovine Sperm Cryopreservation

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**Abstract**

The aim of this study was to evaluate the effects of supplementing different concentrations of EBN into Tris (Tr) and Bioxcell (Bx) extenders on bull sperm cryopreservation. A total of 12 semen samples were collected from mature bulls by electro ejaculation. The semen samples were evaluated both freshly and after cryopreservation for quality based on % sperm general and progressive motility (under a microscope), viability and abnormal morphology (using eosin-nigrosin stain). The fresh samples were then diluted and extended using the two extenders containing 0% (control), 0.03%, 0.06%, and 0.12% of EBN. Chilled at 4°C for 3 hours before packaged into 0.25 mL straws and frozen into liquid nitrogen (-196°C) for 48 hours. Results for both extenders revealed insignificant differences ( $P > 0.05$ ) in all parameters between the different EBN treatment groups and control. Although not significant, 0.12% EBN in both extenders showed the lowest % abnormality, close to the fresh sample reading. In conclusion, EBN concentrations used in this study do not significantly improve sperm quality after freezing. However, the improvement in sperm morphology observed at 0.12% EBN (highest concentration) might imply importance of further increase in dosage for significant effect in future studies.

**INTRODUCTION**

Semen cryopreservation is a biotechnology to preserve and store sperm for a short or long time for many purposes such as in assisted reproduction technologies (ART), species or breed conservation and clinical medicine. However, the quality and life-span of frozen-thawed semen reduces from its fresh quality [3]. Antioxidants are endogenously present in the seminal plasma of the bull [6]. However, when the production of ROS exceeds the antioxidant capacity of the seminal plasma during cryopreservation, it leads to oxidative stress which is harmful to sperm that results in loss of sperm motility and viability.

Edible-bird's nest (EBN) is a dried glutinous secretion from salivary glands of several different swiftlet species (mainly from *Aerodramus fuciphagus*) and contains 35.80% of protein and 46.47% of carbohydrate (9% sialic acid, 7.2% galactosamine, 5.3% glucosamine, 16.9% galactose, and 0.7% fructose). In addition, EBN also contains serine (4.54%) as major amino acid which believed has antioxidant activity and sialic acid also can act as antioxidant [4]. The strong antioxidant property of EBN

reported [11,9], is another potential role and research interest from which both in vivo and in vitro reproductive process are expected to benefit, through reduction of oxidative stress. Oxidative stress (OS), which occurs when oxidants outnumber antioxidants in tissues or cells causing pathological effects, is known to play a role in the pathophysiology of infertility [1]. Thus, being rich in essential nutrients and antioxidant activity, EBN is believed to have a positive effect in maintaining sperm quality during cryopreservation. Hence, this study was designed to determine the effect of EBN in Tris and Bioxcell extenders on quality of bull sperm after cryopreservation.

**MATERIALS AND METHODS**

Twelve semen samples were collected from seven sexually matured bulls in Ladang 16, Taman Pertanian Universiti (TPU), Universiti Putra Malaysia using electro ejaculator. The volume, colour and concentration of every fresh sample was recorded and semen quality evaluation was done prior dilution in Tris and Bioxcell extenders that contain 0% (control), 0.03%, 0.06%, and 0.12% of edible-bird nest (EBN). The diluted semen were then

chilled at 4°C for 3 hours before packaged into 0.25 mL straws and frozen into liquid nitrogen (-196°C) for 48 hours. After 48 hours, three straws were randomly selected from each treatment groups (including control) and thawed in 37°C water bath for 30 seconds for evaluation. Semen quality parameters used for evaluation include general and progressive motility (using light microscope), viability and abnormal morphology (using eosin-nigrosin stain) such as bent tail, coil tail or dag defect, tailless, decapitated and head abnormalities. The semen collection and analyses were conducted according to Khumran et al., [7]. All the data were analyzed using one-way analysis of variance (ANOVA) and the differences among means were tested for significance by Tukey test in which the value of  $P < 0.05$  was considered to be having a significant statistical difference.

## RESULTS AND DISCUSSION

Results for both extenders revealed insignificant differences ( $P > 0.05$ ) in all parameters between the different EBN treatment groups and control (Tables 1,2). Although not significant ( $P > 0.05$ ), 0.12% EBN in both Tr and Bx extenders showed the lowest % abnormality compared with other treatments, which was close to the fresh sample reading (Tables 1,2). According to Bansal & Bilaspuri [2], reactive oxygen species (ROS) increase and antioxidant level decrease during cryopreservation, thus when the sperm exposed to the cold shock and atmospheric oxygen during cryopreservation, lipid peroxidation might occur that results to the damage of sperm plasma membrane. This subsequently can lead to loss of sperm motility, loss in membrane and morphological integrity, impaired cell functions, and induction of sperm apoptosis.

The observed insignificant difference in sperm abnormality between the post-thaw quality and the fresh sample especially

at 0.12% EBN treatment might indicate the role of EBN in protecting sperm morphology from cryo damage. Although no previous study on in vitro testing of EBN as enrichment to bovine semen extenders for sperm cryopreservation, recent studies have shown encouraging results and significant improvement of semen quality after thawing by in vitro addition of supplements such as antioxidant butylated hydroxyl toluene - BHT into Bx extender [7]. EBN is known for its strong antioxidant effect in other studies [11,9]. EBN also contains high amount of sialic acid which is an important molecule found in all animal cells with a number of biological functions including cell communication and signalling, as structural and functional component of the mature testis [5]. Sialic acid which is also found exclusively localized in the sperm acrosomal membrane and head plasma membrane is known to play an important role in the process of fertilization in both human [8] and bovine sperm-zona pellucida (sp-zp) binding process [10].

Moreover, an in vivo study in male castrated rats treated with different concentrations of EBN also reported to demonstrate significant increase in prostate and seminal vesicle indexes, and the protein expression of endothelial nitric oxide synthase (eNOS) implying potential of EBN to promote sexual function. In addition, although not significant compared to the untreated control, an increase in the hormone testosterone and LH levels across treatment groups in a dose dependant manner were also reported by the same study. Composition analysis of EBN by another study has also indicated presence of hormones including testosterone, estradiol, progesterone, LH, FSH and prolactin in EBN.

## CONCLUSION

In conclusion, the present study demonstrated that EBN

**Table 1:** Mean and standard deviation of fresh and post-thawed semen quality parameters processed using Bioxcell extender (Bx) treated with different concentrations of EBN.

Parameters	Fresh Semen	Treatment Groups			
		Bx0	Bx1	Bx2	Bx3
General Motility	82.7 ± 10.4 <sup>a</sup>	32.2 ± 17.7 <sup>b</sup>	32.4 ± 17.9 <sup>b</sup>	29.4 ± 17.3 <sup>b</sup>	30.6 ± 19.7 <sup>b</sup>
Progressive Motility	80.7 ± 13.2 <sup>a</sup>	17.7 ± 20.8	17.6 ± 23.2	15.9 ± 20.2	15.6 ± 23.7 <sup>b</sup>
Viability	69.0 ± 8.2 <sup>a</sup>	39.4 ± 14.3 <sup>b</sup>	37.7 ± 14.8 <sup>b</sup>	39.4 ± 14.8 <sup>b</sup>	40.5 ± 13.8 <sup>b</sup>
Abnormalities	5.7 ± 1.8	7.9 ± 4.6	8.3 ± 5.9	8.2 ± 5.3	6.9 ± 4.1

- Values in the same row with different superscripts (ab) are significantly different at  $\alpha=0.05$
- Bx0: Bioxcell with 0.00% EBN (control); Bx1: Bioxcell with 0.03% of EBN; Bx2: Bioxcell with 0.06% of EBN; Bx3: Bioxcell with 0.12% of EBN

**Table 2:** Mean and standard deviation of fresh and post-thawed sperm quality parameters processed using Tris extender (Tx) treated with different concentrations of EBN.

Parameters	Fresh Semen	Treatment Groups			
		Tr0	Tr1	Tr2	Tr3
General Motility	82.7 ± 10.4 <sup>a</sup>	32.3 ± 12.2 <sup>b</sup>	32.6 ± 15.9 <sup>b</sup>	32.9 ± 16.8 <sup>b</sup>	30.8 ± 17.6 <sup>b</sup>
Progressive Motility	80.7 ± 13.3 <sup>a</sup>	18.8 ± 21.38	16.43 ± 24.1 <sup>b</sup>	17.9 ± 20.2	17.3 ± 15.3
Viability	69.0 ± 8.2 <sup>a</sup>	43.6 ± 12.6 <sup>b</sup>	43.0 ± 15.5 <sup>b</sup>	45.4 ± 15.1 <sup>b</sup>	41.1 ± 17.6 <sup>b</sup>
Abnormalities	5.7 ± 1.8	9.6 ± 6.7	9.0 ± 5.8	9.2 ± 6.7	7.3 ± 3.4

- Values in the same row with different superscripts (ab) are significantly different at  $\alpha=0.05$
- Tr0: Tris with 0.00% of EBN (control); Tr1: Tris with 0.03% of EBN; Tr2: Tris with 0.06% of EBN; Tr3: Tris with 0.12% of EBN

concentrations used in this experiment are not good enough to result in significant improvement in post-thaw semen quality. However, it is worth mentioning that no adverse effect of adding EBN hydrolysates is observed. On the other hand, the minimal improvement in sperm morphology observed at 0.12% EBN treatment (highest dose) might imply the importance of increasing the doses in future investigations.

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### Cite this article

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