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Efficacy of *Nigella sativa* extract on abnormalities of cryopreserved sperm of Holstein bulls raised in Iraq

Naseer H. M. Alrubaie

Department of Surgery and Obstetrics, College of Veterinary Medicine/ University of Baghdad / Baghdad-Iraq

Corresponding author email: naseer.hammoud1202d@covm.uobaghdad.edu.iq

Alyasiri, E. A.

Department of Surgery and Obstetrics, College of Veterinary Medicine/ University of Baghdad / Baghdad-Iraq

Mohammed, O. A.

Department of Artificial Insemination, Ministry of Agriculture Directorate General of Animal Resource / Baghdad-Iraq

Abstract--The present study was performed on Holstein bulls at the Department of Artificial Insemination center/ Directorate of Animal Resource, Ministry of Agriculture in Abu-Ghraib, Baghdad, during the period from December 2021 to June 2022. The study was conducted to enrich low semen quality by adding *Nigella sativa* aqueous extract to semen Tris extender. Three to five years old bulls (No= 4), weighing 500-750 Kg body weight were used for semen collection by artificial vagina (AV) weakly. Bad pooled fresh semen was divided into four groups. First group used a control containing Tris extender only (C), the second group added 0.5% NS extract, third group added 2% NS extract, and the fourth group added 4% NS extract. The results revealed different aqueous extract of N.S after zero time and 2 hrs. Post cooling, for head abnormalities no significant differences ($P<0.05$) between treatment and control, while it recorded a significant decrease in T1 and T2, compared with T3 and T4, after 4 hrs., post cooling and 48 hrs., post freezing (cryopreservation), mid-piece abnormalities reported no significant differences ($P<0.05$) in all groups at zero time, after 4 hrs., post cooling and 24 hrs., post freezing while it recorded a significant decrease ($P<0.05$) in T2 and T3 compared with T1 and T4 after 2hrs. Post cooling, the tail abnormalities recorded significantly lower ($P<0.05$) in T1 and T2 compared with T3 and T4 in different aqueous extract of N.S after zero time, after 2hrs. Post cooling the tail abnormalities reported a

significant decrease ($P<0.05$) in T1 and T4 compared with T3 and T4, as after 4 hrs of cooling a significant decrease ($P<0.05$) in T1 compared with T2, T3 and T4 and after 48 hrs. Post freezing (cryopreservation), the tail abnormalities recorded a significant decrease ($P<0.05$) in T1 and T2 compared with T3 and T4.

Keywords---sperm abnormalities, post cooling, post freezing, *Nigella sativa*.

Introduction

The improvement in quality of semen is an important aspect for maximum utilization of genetically superior sub-fertile sires (Eidan et al., 2017). Sperm abnormalities may cause infertility in different ways, some abnormal sperms might not successfully complete the fertilization procedure, and these abnormalities could affect sperm movement, such as midpiece defect, tail abnormalities and misshapen heads (Mathevon et al., 1998). Sperm morphology has a considerable positive relationship with fertility (Olivira et al., 2013). Noakes et al., (2009) illustrated two types of abnormalities according to the situation of occurrence, the Primary abnormalities which take place during spermatogenesis and as well result from a pathological activity in the testicles and Secondary abnormalities, which took place after sperms leave the testicles. The general sperm morphology is dependent either on spermatogenesis (Graham and Moce, 2005; Ethaeb et al., 2021) or after spermiation due to bad handling or missteps during semen processing, that could lead to twisting of tail or acrosome damage (Chenoweth, 2005). Due to the possible overproduction of ROS during semen preservation, the addition of antioxidant compounds to the semen extender is common. For sperm cryopreservation, antioxidants can be added to the extender before freezing or after semen thawing (Amidi et al, 2016 and Moradi et al, 2020). The use of plants as medicines dates from the earliest years of man's evolution (Miller et al, 2004). Among the promising medicinal plants, *Nigella sativa*, the seeds of *Nigella sativa* are.

The source of the active ingredients of this plant, it is the black seed referred to by the prophet Mohammed as having healing powers (Al-Zubiady, 2007). *N. sativa* seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids (Mohammed et al, 2009; Alyasiri et al, 2015 and Assi et al, 2016). Monosaccharides in the form of glucose, rhamnose, xylose, and arabinose are also found. *Nigella sativa* seeds are rich in the unsaturated (linoleic and oleic acid) and essential fatty acids (Al-Attar and Al-Taisan, 2010). The seeds contain carotene (Al-Okaily et al, 2012) calcium, iron, and potassium (Ayed and Talal, 2011). Some researchers have found that *Nigella sativa* seeds are used for improvement of infertility in male rats (Al-Jishi and Abuo Hozafa, 2000; Al-Mayali, 2007). They have studied the effect of alcoholic extracts of *Nigella sativa* seeds and microbial phytase enzyme on fertility of male rats treated with cadmium chloride and noted that *N. sativa* extract caused enhancement of testes histological function with decrease in the sperm's abnormalities. Alcoholic extracts of *N. sativa* led to activation of reproductive performance in male rats

with decrease of sexual desire time and increase of concentration viability of sperms and decrease of sperm abnormalities (Al-Zubiady, 2007).

Materials and Method

The present study was performed on Holstein bulls at the Department of Artificial Insemination center/ Directorate of Animal Resource, Ministry of Agriculture in Abu-Ghraib, Baghdad, during the period from December 2021 to June 2022. The study was conducted to enrich low semen quality by adding *Nigella sativa* aqueous extract to semen Tris extender. Three to five years old bulls (No= 4), weighing 500-750 Kg body weight were used for semen collection by artificial vagina (AV) weakly. Bad pooled fresh semen was divided into four groups. First group used a control containing Tris extender only (C), the second group added 0.5% NS extract, the third group added 2% NS extract, and the fourth group added 4% NS extract. The studied parameter was Sperm's abnormalities percentage including head, mid piece, and tail of sperm. Plants seeds were purchased from a local market in Baghdad and washed with distal water, and then dried at 50 C° and crushed in a mortar with pestle, a mixture of 10 g seed powder and 50ml distal water were prepared and vortexed for 15-20 min. After equilibration of 30min, centrifugation was carried out at 1340g for 15 min, the supernatant then separated and filtered with filter paper, centrifugation and filtration were repeated twice. The final extracts had been kept frozen at 4 C° until use.

Results and discussion

Table 1. showed the type of sperm abnormalities (head abnormalities) in different aqueous extract of *Nigella Sativa* after zero time, it recorded 6.27 ± 0.73 , 6.49 ± 0.73 , (7.15 ± 0.78) and (7.14 ± 0.73) in T1, T2, T3 and T4 respectively with no significant different ($P < 0.05$) between treatment and control. The head abnormalities reported (6.75 ± 0.65) , (7.3 ± 0.63) , (7.02 ± 0.68) and (7.1 ± 0.63) in T1, T2, T3 and T4 respectively with no significant different between groups after 2hrs post cooling, head abnormalities in T1, T2, T3 and T4 after 4hrs. of cooling was (7.08 ± 0.64) , (7.22 ± 0.61) , (8.43 ± 0.72) and (7.9 ± 0.71) respectively recording a significant decrease in T1 and T2, compared with T3 and T4, after 48hrs post freezing (cryopreservation) recording (8.3 ± 0.77) , (8.94 ± 0.76) , (10.7 ± 0.85) and (9.9 ± 0.72) in T1, T2, T3 and T4 in head abnormalities with a significant decrease in T1 and T2 ($P < 0.05$) compared with T3 and T4 as well as there is no significant differences between T2, T3 and T4. this result agreed with (Parkinson, 2004 and Menon et al., 2011) that it has been generally accepted that bull semen classified as satisfactory should contain at least 70% morphologically normal sperm, with no more than 20% of sperm with an abnormal head. A significant increase in head abnormalities recorded after 24hrs. Post freezing in comparison with post cooling time in all groups, our observation was consistent with a previous report that the most common abnormality was sperm tail abnormality found in bull semen, regardless of breed (Barth and Oko, 1989). Of the head abnormalities encountered in this study, detached head was the most common. In the semen of bulls with acceptable semen quality, it was common to find a small percentage of detached sperm heads, similarly, an average prevalence of 5.3% detached heads was found in 1001 western Canadian range bulls (Barth and Oko, 1989; Gharban

and Al-Shaeli, 2021). In the semen of bulls with acceptable semen quality, it was common to find a small percentage of detached sperm heads, similarly, an average prevalence of 5.3% detached heads was found in 1001 western Canadian range bulls (Barth and Oko, 1989). The use of *Nigella sativa* in low concentrations (0.5% and 2%) led to decreased sperm abnormalities as compared with control after 48 hrs. cryopreservation and this may contribute to that *Nigella sativa* seeds are a good source of iron, calcium, potassium, sodium, copper, and zinc that contribute to sperm maturation and also act as cofactors for functioning of several enzymes that protect semen (El-Battawy and Riad, 2011 and Desai et al., 2015). Table 2. Revealed mid piece abnormalities in different aqueous extract of N.S after zero time, it recorded no significantly ($P < 0.05$) between groups which were (0.0 ± 0.00) , (0.0 ± 0.00) , (0.25 ± 0.47) and (0.15 ± 0.63) in T1, T2, T3 and T4 respectively, and it was (0.4 ± 0.67) , (0.0 ± 0.00) , (0.0 ± 0.00) and (0.7 ± 0.63) in the 4 groups respectively after 2hrs. post cooling recording a significant decrease ($P < 0.05$) in T2 and T3 compared with T1 and T4, after 4hrs. post cooling it was (0.0 ± 0.00) for T1, T2 and T3 and (0.25 ± 0.61) in T4 with no significant differences ($P < 0.05$) between groups, while M .P abnormalities after 48hrs post freezing (cryopreservation) reported (0.0 ± 0.00) , (0.05 ± 0.48) , (0.2 ± 0.61) and 0.35 ± 0.63 in the four groups respectively with no significant differences ($P < 0.05$) between all groups. In a previous study, direct supplementation of *Nigella sativa* extract in extender improved the sperm abnormalities as well as DNA integrity in rabbit (El Battawy & Riad, 2011), whereas others have reported improvement in semen quality with dietary supplementation of *Nigella sativa* seeds/extracts in white rats (Al Sa'aidi et al., 2009), New Zealand rabbit (El-Tohamy et al., 2010), and human males (Marbat et al., 2013) and agreed with (Darand et al., 2019) who recorded that administration of *Nigella sativa* caused a significant increase in spermatid count and motility and a decrease in dead and abnormal sperms. The tail abnormalities recoded significantly lower ($P < 0.05$) in T1 (5.58 ± 0.78) , T2 (6.62 ± 0.85) compared with T3 (7.30 ± 0.74) and T4 (7.35 ± 0.72) in different aqueous extract of N.S after zero time (table 3.), after 2hrs. post cooling the tail abnormalities reported (6.3 ± 0.68) , (6.7 ± 0.80) , (9.1 ± 0.73) and (8.3 ± 0.72) in T1, T2, T3 and T4 respectively with a significant decrease ($P < 0.05$) in T1 and T4 compared with T3 and T4. Tail abnormalities in T1, T2, T3 and T4 after 4hrs. of cooling was (7.47 ± 0.77) , (8.7 ± 0.82) , (9.75 ± 0.61) , (9.07 ± 0.74) respectively in T1, T2, T3 and T4 with a significant decrease ($P < 0.05$) in T1 compared with T2, T3 and T4 as well as after 48hrs. post freezing (cryopreservation), the tail abnormalities recoded (9.27 ± 0.76) , (10.4 ± 0.83) , (11.35 ± 0.76) and (11.45 ± 0.80) in T1, T2, T3 and T4 respectively with a significant decrease ($P < 0.05$) in T1 and T2 compared with T3 and T4. These results agreed with (Hala, 2011) that protective effects of *N. sativa* have been expressed for oxidative status, superoxide anion scavenger, direct cytoprotective effects and indirect antioxidant and androgen activities that protect sperms.

Table 1. Abnormalities of sperms (Head) in different aqueous *extract of Nigella sativa* after 0, 2 and 4hrs. post cooling and 24hrs. post freezing in Holstein bulls born in Iraq

Treatments	post cooling at zero time	post cooling at 2hrs time	post cooling at 4hrs time	Post freezing at 24hrs.
T1	0.0 ± 0.00 B a	0.4 ± 0.67 B a	0.0 ± 0.00 B a	0.0 ± 0.00 C a
T2	0.0 ± 0.0 B a	0.0 ± 0.00 B b	0.0 ± 0.0 B a	0.05 ± 0.48 C a
T3	0.25 ± 0.47 B a	0.0 ± 0.00 C b	0.0 ± 0.00 B a	0.2 ± 0.61 B a
control	0.15 ± 0.63 B a	0.7 ± 0.63 C a	0.25 ± 0.61 C a	0.35 ± 0.63 C a

Different small letters mean significant differences ($P < 0.05$) among groups.

Different capital letters mean significant differences ($P < 0.05$) with in group.

Table 2. Abnormalities of sperms (Mid piece) in different aqueous extract of *Nigella sativa* after 0, 2 and 4hrs. post cooling and 24hrs. post freezing in Holstein bulls born in Iraq

Treatments	post cooling at zero time	post cooling at 2hrs. time	post cooling at 4hrs. time	Post freezing at 24hrs.
T1	6.27 ± 0.73 B a	6.75 ± 0.65 B a	7.08 ± 0.64 B b	8.3 ± 0.77 A b
T2	6.49 ± 0.73 B a	7.3 ± 0.63 B a	7.22 ± 0.61 B b	8.94 ± 0.76 A ab
T3	7.15 ± 0.78 B a	7.02 ± 0.68 B a	8.43 ± 0.72 B a	10.7 ± 0.85 A a
control	7.14 ± 0.73 B a	7.1 ± 0.63 B a	7.9 ± 0.71 B a	9.9 ± 0.72 A a

Different small letters mean significant differences ($P < 0.05$) among groups.

Different capital letters mean significant differences ($P < 0.05$) with in group.

Table 3. Abnormalities of sperms (Tail) in different aqueous extract of *Nigella sativa* after 0, 2 and 4hrs. post cooling and 24hrs. post freezing in Holstein bulls born in Iraq

Treatments	post cooling at zero time	post cooling at 2hrs. time	post cooling at 4hrs. time	Post freezing at 24hrs.
T1	5.58 ± 0.78 A b	6.3 ± 0.68 A b	7.47 ± 0.77 A b	9.27 ± 0.76 A b
T2	6.62 ± 0.85 A b	6.7 ± 0.80 A b	8.7 ± 0.82 A a	10.4 ± 0.83 A b
T3	7.30 ± 0.74 A a	9.1 ± 0.73 A a	9.75 ± 0.61 A a	11.35 ± 0.76 A a
control	7.35 ± 0.72 A a	8.3 ± 0.72 A a	9.07 ± 0.74 A a	11.45 ± 0.80 A a

Different small letters mean significant differences ($P < 0.05$) among groups.

Different capital letters mean significant differences ($P < 0.05$) with in group.

Conclusion

Nigella sativa aqueous extract possess strong antioxidant activity and it decreases the abnormalities in bull spermatozoa at all stages of cryopreservation (post-dilution, post-cooling, and post-thaw) in all concentrations

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