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Efficacy of Nigella sativa extract on individual activity and viability of cryopreserved sperm of Holstein bulls raised in Iraq

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Abstract---The present study was performed on Holstein bulls at the Department of Artificial Insemination pertaining to the Directorate of Animal Resource, Ministry of Agriculture, Abu-Ghraib, Baghdad, during the period from December 2021 to June 2022. The experiments were conducted to improve low semen quality by addition of Nigella sativa aqueous extract to semen Tris extender. Four bulls, 3-5 years old and 500-750 Kg live body weight were trained to semen collection using artificial vagina (AV) weakly bad pooled fresh semen was divided into four groups. First group using as a control containing Tris extender only (C), second group added 0.5% NS extract, third group added 2% NS extract, forth group added 4% NS extract. The results revealed the Individual motility of sperm in different aqueous extract of N.S after cooling (5⁰) at Zero time, 2hrs and 4hrs which recorded a significant differences ($P<0.05$) in T1 compared with other groups as well as in T2 and T4 compared with T3 and after semen cryopreservation for 48hrs it showed that T1 was significantly different ($P<0.05$) from other groups and no significant differences recorded between T2 and T4 (control), as well as it recorded significant differences ($P<0.05$) between T2 and T4 compared with T3. The viability of sperms recorded a significant increase ($P<0.05$) in T1 and T2 compared with T3 and T4, and after 2hrs of cooling the viability of sperms was significantly difference ($P<0.05$) in T1 compared with T2, T3 and T4. The viability of sperms after 4hrs of cooling showed a

significant increase in T1 and T2 ($P < 0.05$) as compared with T3 and T4, while the viability of sperms after 48hrs post freezing (cryopreservation) were significantly increased ($P < 0.05$) in T1 compared with other groups as well as between T2 (2% N.S) compared with T3 and T4. In conclusions, *Nigella sativa* aqueous extract possess strong antioxidant activity and when added in tris extender, it improved the progressive motility and viability and in bull spermatozoa at all stages of cryopreservation (post-dilution, post-cooling, and post-thawing and concentration of 0.5% of this extract was more effective than other concentrations (2% and 4%).

Keywords---*Nigella sativa*, bull, semen characteristics, antioxidants.

Introduction

Good quality semen samples are essential for success in reproductive biotechnologies such as artificial insemination (AL-Badry et al., 2016). For this, it is necessary to properly maintain sperm viability through cryopreservation processes (dilution, refrigeration, freezing, storage, and thawing), especially seminal dilution (Chaveiro et al., 2006). During the cryopreservation process of semen samples, there is continuous and excessive production of reactive oxygen species (ROS), which are harmful to sperm, as it induces lipoperoxidation, apoptosis, DNA damage with a consequent reduction in sperm quality, since the sperm membrane has high concentrations of lipids and proteins that are highly susceptible to ROS (Asma-ul-husna et al., 2017). On the other hand, low concentrations of ROS are responsible for the regulation of physiological events in the cells (Kothari et al., 2010). Antioxidant substances have been added to diluent media in order to minimize the harmful effects caused by ROS on sperm of various species, including the bovine. Antioxidants such as agglutination and melatonin slow down the rate of oxidation by maintaining ROS production at physiological levels (Ashok et al., 2014; Silva, 2020; Gharban and Al-Shaeli, 2021), and its replacement by many plant-based products to maintain sperm viability (Forouzanfar et al., 2010) is currently being evaluated. Medicinal plants serve as therapeutic alternatives, safer choices, or in A larger number of these plants and their extract have shown beneficial therapeutic effects, including antioxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects (Salem and Hossain, 2000; Huffman, 2003; Miller et al, 2004 and Alyasiri et al, 2015). Among the promising medicinal plants, *Nigella sativa*, a dicotyledonous of the Ranunculaceae family, is an amazing herb with a rich historical and religious background (Goreja, 2003). *Nigella sativa* oil was characterized by its anti-oxidative actions to counteract the impairment that occur in the epididymal sperm characters caused by hydrogen peroxide (H_2O_2) treatment (Tawfeek et al, 2006). So, the study aimed to study the effect of three different concentrations of *Nigella Sativa* as additive to semen diluent on some physical properties (individual activity and viability) of bull semen at different steps of frozen semen processing (after cooling and freezing).

Materials and Methods

The present investigation was performed on Holstein bulls at the Department of Artificial Insemination pertaining to the Directorate of Animal Resource, Ministry of Agriculture, Abu-Ghraib, Baghdad, during the period from December 2021 to June 2022. The experiments were conducted to improve low semen quality by addition of *Nigella sativa* aqueous extract to semen Tris extender. Four bulls, 3-5 years old and 500-750 Kg live body weight were trained to semen collection using artificial vagina (AV) weakly. bad pooled fresh semen was divided into four groups. First group using as a control containing Tris extender only (C), second group added 0.5% NS extract, third group added 2% NS extract, forth group added 4% NS extract. The studied parameter was Sperm's cell individual motility and live sperm percentage. Plants seeds were purchased from a local market in Baghdad and washed with distal water, and then dried at 50 Co 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 30 min, 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 Co until use (Awan et al, 2018).

Results and Discussion

The results in table 1. revealed the Individual motility of sperm in different aqueous extract of N.S after cooling (5°) at Zero time which recorded (43.3±1.25), (35.5±1.24), (30.5±1.21) and (38.0±1.40) in T1 (0.5 % N.S), T2 (2% N.S), T3(4% N.S), and T4 (control) respectively, with a significant differences (P<0.05) in T1 compared with other groups as well as in T2 and T4 compared with T3. The Individual motility after 2hrs of cooling reported (43.5±1.25), (35.5±1.24), (30.5±1.21) and (36.5±1.36) in T1, T2, T3 and T4 (control) respectively, a significant differences (P<0.05) recorded between T1 and other groups, also there was significant differences (P<0.05) between T2 and control comparison with T3. The results in table 1. also revealed individual motility of sperm with different concentration of N.S (43.5±1.25), (35.5±1.24), (30.5±1.21) and (36.0±1.35) in T1 ,T2, T3 and T4 respectively after 4hrs of cooling, which showed a significant differences (P<0.05) between T1 and other groups as well as between T2 and T4 with T3 and no significant differences between T2 and T4 was recorded and after semen cryopreservation for 48hrs it recorded (35.5 ±1.34), (28.5±1.22), (23.5±1.17) and (27.5±1.30) for T1, T2, T3 and T4 respectively, T1(0.5% N.S) was significantly different (P<0.05) from other groups and no significant differences recorded between T2 and T4 (control), as well as it recorded significant differences (P<0.05) between T2 and T4 compared with T3. these results agreed with (El-Battawy and Riad, 2011) that direct supplementation of *Nigella sativa* extract in extender improved the sperm motility in rabbit, and with (Awan et al., 2018) who showed an increased pattern of sperm motility at low concentrations of *Nigella sativa* extract (1%–4%) while at higher concentrations (5% and 6%), sperm motility. This difference in sperm motility might have been due to thymoquinone that exhibits antioxidant properties at low concentrations that protect spermatozoa from ROS versus pro-oxidant properties at high concentrations that enhances the production of ROS and ultimately results in reduced sperm motility (Miah et al., 2020).

Table (1) Individual motility of sperms in different aqueous extract *Nigella sativa* after cooling (5^o) at zero, 2hrs, 4hrs and 48 hrs. post freezing in Holstein bulls born in Iraq

period Treatment	Individual motility after cooling at 5 zero time	Individual activity at cooling after 2hrs	Individual activity at cooling after 4hrs	Individual activity after 48hrs post c
T1	43.3± 1.25 a	43.5± 1.25 a	43.5± 1.25 a	35.5 ± 1.34 a
T2	35.5 ± 1.24 b	35.5 ± 1.24 b	35.5 ± 1.24 b	28.5 ± 1.22 b
T3	30.5 ± 1.21 c	30.5 ± 1.21 c	30.5 ± 1.21 c	23.5 ± 1.17 c
T4 (control)	38.0 ± 1.40 b	36.5± 1.36 b	36.0 ± 1.35 b	27.5 ± 1.30 b

Different small letters mean significant differences (p <0.05) among groups of treatment

In Table 2, the findings reported (73.53 ±1.35), (72.26±1.18), (69.4±1.22) and (70.56±1.26) in T1(0.5%, N.S), T2 (2% N.S), T3 (4% N.S) and T4(without treatment) respectively at zero time in Holstein bulls born in Iraq. A significant increase (P<0.05) revealed in T1 and T2 compared with T3 and T4. After 2hrs of cooling the viability of sperms (table 2) was (72.73±1.27), (69.95±1.19), (67.25±1.19), and (67.95±1.28) in the fourth groups respectively recording a significant differences (P<0.05) for T1 in comparison with T2, T3 and T4. The results reported the viability of sperms After 4hrs of cooling in T1 (0.5%, N.S), T2 (2% N.S), T3 (4% N.S) and T4 (without treatment) which were (71.0±1.24), (68.54±1.18), (65.83±1.19), and (66.2±1.31) respectively. There was a significant increase in sperm viability in T1 and T2 (P<0.05) as compared with T3 and T4 as well as no significant differences (P<0.05) was recorded between T2, T3 and T4. While the viability of sperms after 48hrs post freezing (cryopreservation) were (64.2±1.32), (60.0±1.29), (55.25±1.29), and (55.65±1.33) in T1, T2, T3 and T4 respectively, and a significant increase (P<0.05) was recorded between T1 and other groups as well as between T2 (2% N.S) compared with T3 and T4. These results agreed with (El-Battawy and Riad, 2011) that direct supplementation of *Nigella sativa* extract in extender improved the sperm viability and DNA integrity in rabbits, it also agreed with (Awan et al., 2018) who reported that sperm viability (live spermatozoa with intact acrosome) in cryopreserved semen was significantly higher (p < .05) in extender supplemented with *Nigella sativa* extract compared to control.

Table 2. Viability (Live) of sperms in different aqueous extract of *Nigella sativa* after cooling (5°) at zero time, 2hrs, 4hrs and 48hrs. post freezing in Holstein bulls born in Iraq

Treatment	Viability (Live) after cooling at zero time	Viability (Live) after cooling at 2hrs time	Viability (Live) after cooling at 4hrs time	Viability (Live) after cooling at 4hrs time
T1	73.53 ± 1.35 a	72.73 ± 1.27 a	71.0 ± 1.24 a	64.2 ± 1.32 a
T2	72.26 ± 1.18 a	69.95 ± 1.19 b	68.54 ± 1.18 ab	60.2 ± 1.29 b
T3	69.4 ± 1.22 b	67.25 ± 1.19 b	65.83 ± 1.19 b	55.25 ± 1.29 c
T4(control)	70.56 ± 1.26 b	67.95 ± 1.28b	66.2 ± 1.31 b	55.65 ± 1.33 c

Different small letters mean significant differences (p <0.05) among groups of treatment

In conclusions, *Nigella sativa* aqueous extract possess strong antioxidant activity and when added in trisextender, it improved the progressive motility and viability and in bull spermatozoa at all stages of cryopreservation (post-dilution, post-cooling, and post-thaw) and concentration of 0.5% of this extract was more effective than other concentrations (2% and 4%).

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