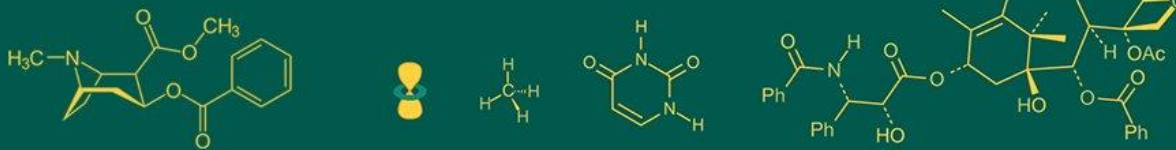

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Effect of black cumin (*Nigella sativa*) seeds extract in tris egg yolk citrate extender on sperm motility of cryopreserved Surti buck semen

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Abstract
The present research work was conducted to examine effect of different concentrations of Black cumin (*Nigella sativa*) seeds extract in Tris egg yolk citrate extender on motility and viability of cryopreserved Surti buck semen. Total 64 semen ejaculates were collected from four Surti buck (16 ejaculates/buck) twice a week by artificial vagina method. Semen samples were diluted with Tris egg yolk citrate extender and Black cumin (*Nigella sativa*) seeds aqueous extract was supplemented at different concentrations *viz.* 0% (T_1), 3% (T_2), 4% (T_3), 5% (T_4) (pH 6.5-6.8) maintaining final concentration of 100×10^6sperm/ml. After making the groups, the semen samples were examined for individual sperm motility (%), Motility Degeneration Rate (MDR) (%) for all groups. Result showed that highest mean individual sperm motility (%) was found at initial, pre-freeze and post-thaw stage in T_3 group followed by T_2, T_4 and T_1 group. Lowest mean motility degradation rate (%) was found at pre-freeze and post-thaw stage in T_3 group followed by T_2, T_4 and T_1 group. It was concluded that post-thawed individual sperm motility and MDR parameters were well maintained at 4% supplementation of Black cumin (*Nigella sativa*) seed extract in Tris egg yolk citrate extender.

Keywords: Cryopreserved Semen, Black cumin (*Nigella sativa*) seed aqueous extract, Motility, Motility Degeneration Rate, Surti buck semen

Introduction

Buck ejaculates are small in volume with a high concentration of spermatozoa (Memon *et al.*, 1986) [1]. Semen is diluted after neat semen evaluation using different kinds of extender. An extender increases the volume of semen and prolongs the life of spermatozoa with fertilizing capacity (Mishra *et al.*, 2010) [2]. Buck semen can be preserved either at room temperature temporarily, at refrigerated temperature for 24- 48 hours (Ferdinand *et al.*, 2012) [3] or cryopreserved (Beltran *et al.*, 2013) [4] for long term storage. The viability of the preserved sperms may be affected by many factors including storage temperature, cryoprotectant concentration, cooling rate, extender composition, free radical contents, seminal plasma contents, and antiseptic factors (Hezavehei *et al.,* 2018) [5]. During the process of cryopreservation, endogenous antioxidant concentration, semen quality and fertility become compromised due to the generation of reactive oxygen species (ROS) (Holt, 2000) [6]. More number of viable and motile spermatozoa is important to maintain quality of cryopreserved semen which can be achieved by minimizing deteriorative factors. Reactive oxygen species (ROS) like hydroxyl, superoxide, nitric oxide, and hydrogen peroxide (H_2O_2) interact with sperm plasma membrane resulting in the occurrence of lipid peroxidation (LPO).

Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.*, 1998) [7] improve seminal attributes. Researchers found that addition of exogenous plant-based extract can reduce the oxidative stress caused by ROS. Such a one plant based extract from seeds of Black cumin (*Nigella sativa*) have same potential to reduce oxidative stress originates during cryopreservation. Black cumin (*Nigella sativa*) is believed to contain essential elements such as thymoquinone, dithymoquinone, 4-terpineol, carvacrol, anethole, thymol, and alpha-pinene (Awan *et al.,* 2018) [8].

Beneficial effects of various forms of Black cumin (*Nigella sativa*) seeds was reported in rats (Mansour *et al.,* 2013) [9]; in rabbits (El-Gindy, 2022) [10] and in cocks (Ezzat *et al.,* 2019) [11]. Black cumin (*Nigella sativa*) seed extract is effective for improving the quality of sperm after vitrification (Nasiri *et al*., 2022) [12]. Looking into the various properties of Black cumin (*Nigella sativa*) seeds extract study has been undertaken on effect of black cumin (*Nigella sativa*) seeds extract in tris egg yolk citrate extender on motility of cryopreserved Surti buck semen.

Materials and Methods

Semen collection, experimental group and cryopreservation

The study was conducted on four apparently healthy mature Surti bucks above one years of age maintained under the All India Coordinated Research Project (AICRP) on Surti Goat at Livestock Research Station, Kamdhenu University Navsari. The selected bucks were housed in a common covered pen and under naturally existing photoperiod and air temperature of the South Gujarat. The selected bucks were housed in a common covered pen and separated from females. The bucks were trained to donate the semen in artificial vagina by using female (doe) as dummy. After completion of the training period of about one month, semen was collected regularly by using artificial vagina twice a week from each buck for up to 8 weeks and total 64 semen ejaculates (16 ejaculates from each buck) were collected.

Semen was collected from all the selected bucks at early morning between 6.30 AM to 7.30 AM with the help of Eight-inch Artificial Vagina (AV) maintaining inner temperature of 40 °C to 42 °C and sufficient pressure. In order to maintain quality of semen, all the parts of artificial vagina are properly sterilized and for each buck separate Artificial vagina was used and during collection buck apron was applied to prevent further contamination. In order to increase the semen volume as well as eliminate individual buck variability the ejaculates of all four bucks were pooled. Only semen samples with initial motility ≥ 70% were considered for further processing. The pooled semen was extended with tris egg yolk citrate extender to achieve final concentration of 100×10^6 sperm/ml.

The diluted semen was separated into four equal aliquots, and each aliquot was treated with different concentrations of Black cumin (*Nigella sativa*) seeds aqueous extract *viz.*0% (control T_1), 3% (T_2), 4% (T_3) and 5% (T_4) (pH 6.5-6.8). According to different groups, extended semen was filled in previously marked 0.5ml French medium straw (IMV Technologies, France) using micropipette having final concentration of 50×10^6 sperm/straw. At least ten straws were prepared for each group. The filled straws were sealed with the help of polyvinyl alcohol powder (HiMedia Laboratories Pvt. Ltd.) and all the loaded straws were laid on a floating rack (Minitube, Germany) and placed in a refrigerator at 4 ˚C for equilibration about 4 hours. After equilibration, the floating rack holding the straws were placed in a manual vapour freezing unit (Minitube, Germany) for 10 minutes in such a way that the straws were remain 5 cm above the liquid nitrogen in vaporous phase. After completion of freezing the straws were directly and quickly plunged into liquid nitrogen container. After making the groups as above, the semen samples were examined for individual sperm motility (%) for all groups at initial, Pre-freeze and at post-thaw (24 hours after cryopreservation) using standard methods. The motility degradation rate (MDR) (%) was calculated as per the formula gave by Campos *et al*., 2004 [13].

Preparation of Black cumin (*Nigella sativa*) seeds aqueous extract

The seeds were carefully washed with clean water to get rid of dust and dirt. They were air shade dried for four days at room temperature and pulverized into a fine powder using mixer grinder. 100 gm of dried seed powder was extracted with water in glass container covered with aluminum foil and allowed to stand at room temperature for a period of 24 hours with frequent agitation until soluble matter had dissolved. The aqueous extract of seeds of Black cumin (*Nigella sativa*) was prepared through liquid partition method. The aqueous extract was stored in vial and kept in a refrigerator at -20 °C prior to sample preparation for subsequent analyses.

Statistical analysis

Descriptive analysis was carried out and mean ± SE was calculated for all the designated groups of neat semen parameters as well as extended semen parameters at various time intervals. The test of significance among the groups for above parameters was made by analysis of variance (ANOVA) and the mean difference between the groups were tested by using Duncan's new Multiple Range test (DNMRT) at 5 and 1 percent level of significance.

Results and Discussions

Individual Sperm motility

The initial mean individual sperm motility percent differed non-significantly between T_1 (80.94±0.94), T_2 (82.50±1.12), T_3 (82.81±0.91) and T_4 (82.19±1.20) groups (Table 1). Pre-freeze mean individual sperm motility (%) was significantly ($p<0.01$) higher in T_3 group (69.38±1.64) as compared to T_2 (63.44±1.87), T_4 (59.69±2.39) and T_1 (54.06±1.95). Pre-freeze mean individual sperm motility (%) was non-significantly differed among T_2 (63.44±1.87) and T_4 (59.69±2.39) groups whereas, it was significantly ($p<0.01$) higher as compared to T_1 group (54.06±1.95).

Post-thaw mean individual sperm motility (%) was significantly ($p<0.01$) higher in T_3 group (46.56±1.35) as compared to T_1 (29.38±1.82), T_2 (40.31±1.74) and T_4 (37.50±2.19) groups. However, post thaw mean individual sperm motility (%) in T_2 (40.31±1.74) and T_4 (37.50±2.19) groups were significantly ($p<0.01$) higher as compared to T_1 (29.38±1.82) group and both groups T_2 (40.31±1.74) and T_4 (37.50±2.19) non-significantly differed with each other. The corresponding overall mean individual sperm motility (%) irrespective of treatment groups was decreased with increasing preservation time at initial (82.11±0.52), pre-freeze (61.64±1.19) and post-thaw (38.44±1.17) stage. The overall mean individual sperm motility (%) in respective of different preservation time differed significantly ($p<0.01$) with each other.

The corresponding overall mean individual sperm motility (%) irrespective of time interval was significantly ($p<0.05$) higher in T_3 (66.25±2.31) group as compared to T_1 (54.79±3.22) group. While T_3 (66.25±2.31) group differed non-significantly with T_2 (62.08±2.67) and T_4 (59.79±2.89) groups. Overall mean individual sperm motility (%) in T_2 (62.08±2.67) and T_4 (59.79±2.89) groups was non-significantly higher as compared to T_1 (54.79±3.22) group.

Table 1: Effect of different concentrations of Black cumin (*Nigella sativa)* seeds aqueous extract on Individual Sperm Motility percent of Surti buck semen at various stages of cryopreservation (Mean ± SE)

s	Individual Sperm Motility (%) (n=16)			Overall (n= 48)	F-value	P-value
	Initial	Pre-freeze	Post-thaw			
T_1	$80.94\pm0.94_x$	$54.06\pm1.95^c{}_y$	$29.38\pm1.82^c{}_z$	54.79 ± 3.22^b	249.97**	0.00
T_2	$82.50\pm1.12_x$	$63.44\pm1.87^b{}_y$	$40.31\pm1.74^b{}_z$	62.08 ± 2.67^{ab}	172.82**	0.00
T_3	$82.81\pm0.91_x$	$69.38\pm1.64^a{}_y$	$46.56\pm1.35^a{}_z$	66.25 ± 2.31^a	189.18**	0.00
T_4	$82.19\pm1.20_x$	$59.69\pm2.39^b{}_y$	$37.50\pm2.19^b{}_z$	59.79 ± 2.89^{ab}	125.16**	0.00
Overall (n=64)	$82.11\pm0.52_x$	$61.64\pm1.19_y$	$38.44\pm1.17_z$	--	2.93*	0.03
F value	0.61	10.56**	15.73**	466.22**	--	--
P value	0.60	0.00	0.00	0.00	--	--

$^{a-c}$ Means with different superscript within a column (between the groups) differs significantly at $p<0.05$. $_{x-z}$ Means with different subscript between a column (between various stages) differs significantly at $p<0.01$. $^{**}p<0.01$.

T_1 - control, T_2 - 3% Black cumin(*Nigella sativa*)seeds aqueous extract, T_3 - 4% Black cumin(*Nigella sativa*) seeds aqueous extract, T_4 - 5% Black cumin(*Nigella sativa*)seeds aqueous extract.

Moreover, mean individual sperm motility (%) in T_1, T_2, T_3 and T_4 groups were significantly ($p<0.01$) higher at initial (80.94±0.94, 82.50±1.12, 82.81±0.91 and 82.19±1.20) stage as compared to pre-freeze (54.06±1.95, 63.44±1.87, 69.38±1.64 and 59.69±2.39) stage and post-thaw (29.38±1.82, 40.31±1.74, 46.56±1.35 and 37.50±2.19) stage. Furthermore, mean individual sperm motility (%) among initial, pre-freeze and post-thaw stage differed significantly ($p<0.01$) in all the groups. The corresponding overall mean individual motility (%) irrespective of treatment groups were significantly ($p<0.01$) reduced with increasing preservation time at initial stage (82.11±0.52) followed by pre-freeze (61.64±1.19) and post-thaw (38.44±1.17) stages.

Highest mean individual sperm motility (%) was found at initial, pre-freeze and post-thaw stage in T_3 (82.81±0.91, 69.38±1.64 and 46.56±1.35) group followed by T_2 (82.50±1.12, 63.44±1.87 and 40.31±1.74) group and T_4 (82.19±1.20, 59.69±2.39 and 37.50±2.19) group. While lowest mean individual sperm motility (%) was found in T_1 group at initial (80.94±0.94), pre-freeze (54.06±1.95) and post-thaw (29.38±1.82) stages. The main compound of Black cumin (*Nigella sativa*) seeds contained higher amount of flavonoids and ascorbic acid. However, many researchers studied the effect of various medicinal plant (coconut water, green tea, honey, Entada abyssinica, moringa leaf etc) extract and as such quercetin, thymoquinone having flavonoids compound. Hence, the discussion was made on that basis.

The findings of present study showed that 4% Black cumin (*Nigella sativa*) seeds aqueous extract supplementation in tris egg yolk citrate extender could prolong the individual sperm motility (%) at pre-freeze (69.38±1.64) and post-thaw (46.56±1.35) stage. In accordance to this finding Awan *et al*. (2018) [8] in buffalo; Miah *et al*. (2018) [14] in ovine; Nasiri *et al.* (2022) [12] in human; El-Battawy and Riad (2011) [15] in rabbit; Inanc *et al.* (2021) [16] in ram; Al-Saaidi *et al.* (2009) [17], Mohammad *et al.* (2009) [18] and Mansour *et al.* (2013) [9] in rats, El-Nattat *et al.* (2019) [19] in rabbit also reported higher sperm motility in lower concentration of *Nigella sativa* supplemented groups as compared to control group.

The highest pre-freeze and post-thaw motility (%) found in T_3 (4% *Nigella sativa* aqueous extract) group followed by T_2 (3% *Nigella sativa* aqueous extract) group in present study, which was compliance with Awan *et al*. (2018) [8], who also found significantly ($p<0.05$) higher sperm motility in post-cooling and post-thaw semen in 4% *Nigella sativa* extract group as compared to control group followed by 3% *Nigella sativa* extract supplemented group in buffalo semen. Nasiri *et al.* (2022) [12] also found similar trend in sperm motility; they observed significantly ($p<0.05$) higher sperm motility in 4% *Nigella sativa* extract supplemented group followed by 3% *Nigella sativa* extract supplemented in human sperm during vitrification. Miah *et al*. (2018) [14] also reported that sperm motility maintained at low concentrations of *Nigella sativa* oil (NSO) supplemented group. They found significantly higher sperm motility (%) in 10 g/ml of NSO supplemented group up to 2 hours of post-thaw incubation. Inanc *et al.* (2021) [16] also reported significantly ($p<0.05$) higher progressive motility in thymoquinone (active compound of *Nigella sativa*) supplementation in extender at 100 µg/ml (TQ 100) followed by in TQ50 supplemented group as compared to control group. They concluded that higher motility was due to positive effect of thymoquinone on aerobic respiration with oxidative phosphorylation enzyme and eliminating free radicals contributing higher motility.

El-Battawy and Riad (2011) [15] found that at 200 µl/ml of *Nigella sativa* supplemented in tris-based extender could preserve sperm motility (%) significantly ($p<0.05$) higher as compared to control group at 2nd day of incubation (5°C). They concluded that this positive effect on motility was due to antioxidant property and rich source of calcium, sodium potassium, iron that are essential for co-enzyme that protects sperm form cryo-damage. Moreover, El-Seadawy *et al.* (2022) [20] observed significantly ($p<0.05$) higher post thawed progressive sperm motility (%) in 0.64 mg/ml group (45.41 ± 0.70), 0.56 mg/ml group (35.32 ± 0.99) and 0.48 mg/ml group (25.08 ± 0.81) as compared to control (30.33 ± 0.62) of moringa leaf extract supplemented group. Ahmed *et al.* (2019) [21] found post thawed stage buffalo bull sperm motility (%) was higher in 200 mM group (40.56 ± 1.55) followed by 150 mM (37.22 ± 1.47) 100 mM (30.56 ± 1.55), 50 mM (31.11 ± 1.11) quercetin supplemented and control (27.78 ± 1.21) group.

Furthermore, oral administration of *Nigella sativa* in various forms and various doses at various duration resulted in significantly ($p<0.05$) higher sperm motility (%) as compared to control group of cauda epididymal sperm stated by Al-Saaidi *et al.* (2009) [17]; Mohammad *et al.* (2009) [18]; Mansour *et al.* (2013) [9] in rats and El-Nattat *et al.* (2019) [19] in rabbit. Al-Saaidi *et al.* (2009) [17] reported oral supplementation of alcoholic extract of *Nigella sativa* at 0.5 and 1.5 g/kg body weight showed significantly ($p<0.01$) higher sperm motility (%) as compared to control group in rats. Similarly, Mansour *et al.* (2013) [9] reported administration of *Nigella sativa* oil at 0.4 ml in rats showed

improved sperm motility (%) as compared to control group. Moreover, El-Nattat *et al.* (2019) [19] also reported oral administration of 3.0 ml and 1.5 ml of *Nigella sativa* water extract in rabbit showed significantly ($p<0.05$) higher sperm motility (%) as compared to control group. Mohammad *et al.* (2009) [18] reported that oral administration of *Nigella sativa* aqueous extract feed at dose rate of 300 mg/kg showed significantly ($p<0.01$) higher epididymal sperm motility (%) in treated group as compared to control group. Whereas, El-Tohamy *et al*. (2010) [22] reported motile sperm (%) was non-significantly lower in Black cumin meal supplemented group (82.56±1.94) as compared to control (82.77±3.55) group in male growing New Zealand rabbit.

This beneficial effect of *Nigella sativa* extract may be due to presence of unsaturated fatty acids (Linoleic acids, palmitic acid, stearic acid, oleic acids) (Nickavar *et al.* 2003) [23] and Thymoquinone that possesses the capability to scavenge superoxide, hydroxyl radical and singlet molecular oxygen (Burits and Bucar, 2000) [24]. Besides that, Black seeds (*Nigella sativa*) contain proteins, vitamins and minerals like zinc, copper, and magnesium (Al-Saaidi *et al.* 2009) [17] that enhances sperm metabolism and maintain its motility. Thymoquinone was also demonstrated as superoxide anion scavenger, direct cryoprotective and indirect antioxidant activities (Hala, 2011) [26].

In context to the present study, pre-freeze and post-thaw sperm motility in T_4 group (5% Black cumin (*Nigella sativa*) seed aqueous extract) was significantly ($p<0.01$) decreased as compared to T_1 and T_3 group and non-significantly decreased as compared to T_2 group. This reverse trend in dose-dependent decrease in sperm motility also observed by Awan *et al*. (2018) [8], they reported that sperm motility was significantly decreased at 5% *Nigella sativa* extract in post-cooling and post-thawed semen as compared to sperm motility at 4% *Nigella sativa* extract in post-cooling and post-thawed semen. Nasiri *et al.* (2022) [12] also reported same sperm motility trend above 4% *Nigella sativa* extract supplemented in extender. They reported non-significantly decreased sperm motility at 5% *Nigella sativa* extract supplemented group as compared to 4% and 3% of *Nigella sativa* extract supplemented group during vitrification of human spermatozoa. Miah *et al*. (2018) [14] also stated same trend in their study. They found significantly ($p<0.05$) higher sperm motility at lower doses (10 g/ml of NSO) as compared to higher dose and control group.

Similar reverse trend in dose-dependent decrease in motility was reported by Khan *et al.* (2017) [27]. They found *Camelia sinensis* (green tea) extract supplemented group showed significantly ($p<0.05$) higher post thaw sperm motility at 0.75% of green tea extract supplemented group as compared to control group in bull spermatozoa, while in 1.00% green tea extract supplemented group, they found significantly ($p<0.05$) lower post thaw sperm motility as compared to control group in bull semen.

In present study, decreased sperm motility may be due to thymoquinone, the active compound of *Nigella sativa*. Thymoquinone has antioxidant properties at low concentrations but causes increase in ROS production at concentrations higher than 4% which leads to reduction in motility (Nasiri *et al.* 2022) [12]. This discrepancy in sperm motility might be due to thymoquinone that exhibits antioxidant properties at low concentrations that protect spermatozoa from ROS versus oxidant properties at high concentrations that enhances the production of ROS and ultimately results in reduced sperm motility (Alenzi *et al.* 2010 [28]; Burits and Bucar, 2000 [24]). Similar trend was also reported by Sobeh *et al.* (2020) [29]. They found post thaw sperm motility significantly ($p<0.05$) increased up to 375 µg/ml of *Entada abyssinica* bark extract supplemented group in ram semen.

Contrasting to present study, Maidin *et al.* (2018) [30] reported initial sperm motility was non-significantly lower at 1.5 and 2 hours after collection of semen in 0.5% *Nigella sativa* oil treated group as compared to control group. They suggested that protective effect of lipid peroxidation in this treatment only protects the sperm cells from oxidative stress activity and protection stops slowly after 2 hours and sperm motility is compromised.

Motility Degradation Rate (%)

The pre-freeze mean motility degradation rate (%) was significantly ($p<0.01$) lower in T_3 (16.27±1.65) group as compared to T_2 (23.24±1.72), T_4 (27.64±2.34) and T_1 (33.23±2.23) group (Table 2). Pre-freeze mean motility degradation rate (%) was significantly ($p<0.01$) lower in T_2 (50.10 ±2.15) group as compared to T_1 (63.77±2.14) group and non-significantly lower as compared to T_4 (53.76±2.63) group. Pre-freeze mean motility degradation rate (%) was non-significantly lower in T_4 (27.64±2.34) group as compared to T_1 (33.23±2.23) group. Post-thaw mean motility degradation rate (%) was significantly ($p<0.01$) lower in T_3 (42.69±1.74) group as compared to T_2 (50.10 ±2.15), T_4 (53.76±2.63) and T_1 (63.77±2.14) groups. While post-thaw mean motility degradation rate (%) in T_2 (50.10 ±2.15) and T_4 (53.76±2.63) groups differed non-significantly with each other and significantly ($p<0.01$) lower as compared to T_1 (33.23±2.23) group.

The corresponding overall mean motility degradation rate (%) irrespective of treatment groups were increased with increasing preservation time at pre-freeze (25.09±1.25) and post-thaw (52.58±1.43) stage. The overall mean motility degradation rate (%) irrespective of different treatment groups were significantly ($p<0.01$) differed among various stages of cryopreservation.

Table 2: Effect of different concentrations of Black cumin (*Nigella sativa*) seeds aqueous extract on Motility Degradation Rate (MDR) percent of Surti buck semen at various stages of cryopreservation (Mean ± SE)

Groups	MDR (%) (n=16)		Overall (n= 32)	F value	P value
	Pre-freeze	Post-thaw			
T_1	$33.23±2.23^{a}{}_{y}$	$63.77±2.14^{a}{}_{x}$	$48.50±3.13^{a}$	0.30**	0.00
T_2	$23.24±1.72^{b}{}_{y}$	$50.10±2.15^{b}{}_{x}$	$36.67±2.77^{bc}$	0.99**	0.00
T_3	$16.27±1.65^{c}{}_{y}$	$42.69±1.74^{c}{}_{x}$	$29.48±2.65^{c}$	0.15**	0.00
T_4	$27.64±2.34^{ab}{}_{y}$	$53.76±2.63^{b}{}_{x}$	$40.70±2.91^{ab}$	0.34**	0.00
Overall (n=64)	$25.09±1.25_{y}$	$52.58±1.43_{x}$	--	7.64**	0.00
F value	12.75**	16.05**	1.93**	--	--
P value	0.00	0.00	0.00	--	--

$^{a-c}$ Means with different superscript within a column (between the groups) differs significantly at $p<0.01$. $_{x-z}$ Means with different subscript between a column (between various stages) differs significantly at $p<0.01$. **$p<0.01$

T_1 - control, T_2 - 3% Black cumin(*Nigella sativa*)seeds aqueous extract, T_3 - 4% Black cumin(*Nigella sativa*) seeds aqueous extract, T_4 - 5% Black cumin(*Nigella sativa*)seeds aqueous extract.

Moreover, mean motility degradation rate (%) in T_1, T_2, T_3 and T_4 groups were significantly lower ($p<0.01$) at pre-freeze (33.23±2.23, 23.24±1.72, 16.27±1.65 and 27.64±2.34) stage as compared to post-thaw (63.77±2.14, 50.10 ±2.15, 42.69±1.74 and 53.76±2.63) stage. Furthermore, mean motility degradation rate (%) among pre-freeze and post-thaw stage was differed significantly ($p<0.01$) in all the groups. The corresponding overall mean motility degradation rate (%) irrespective of time interval was significantly ($p<0.01$) lower in T_3 (29.48 ± 2.65) as compared to T_4 (40.70 ± 2.91) and T_1 (48.50 ± 3.13) group. Overall mean motility degradation rate (%) in T_2 (36.67 ± 2.77) group was significantly ($p<0.01$) lower as compared to T_1 (48.50 ± 3.13) group and non-significantly lower as compared to T_4 (40.70 ± 2.91) group. Lowest mean motility degradation rate (%) was found at pre-freeze and post-thaw stage in T_3 (16.27±1.65 and 42.69±1.74) group followed by T_2 (23.24±1.72 and 50.10 ±2.15) and T_4 (27.64±2.34 and 53.76±2.63) group. While highest mean motility degradation rate (%) was found in T_1 group at pre-freeze (33.23±2.23) and post-thaw (63.77±2.14) stage.

In present study lowest MDR was observed ($p<0.01$) at 4% *Nigella sativa* aqueous extract supplemented in tris-based extender group at pre-freeze and post-thawed semen as compared to control group. While addition at 3% *Nigella sativa* aqueous extract supplementation showed higher MDR rate as compared to 4%*Nigella sativa* aqueous extract added group. Similar pattern of MDR was also observed by Patel *et al.* (2019) [31]; Baldaniya *et al.* (2020) [32] and Lima *et al.* (2013) [33] in buck semen using supplementation of bee's honey and coconut water in tris-based extender. Patel *et al.* (2019) [31] reported that addition of 4% honey in tris-based extender showed lower MDR (24.15 ± 2.74) as compared to control (45.61 ± 2.56) group at 48 hours of refrigerated temperature, while 3% honey supplemented group had higher MDR value than that of 4% honey supplemented group in Surti buck semen. Whereas, Baldaniya *et al.* (2020) [32] reported addition of 5% coconut water in extender showed lowest MDR percentages (24.03 ± 3.74) as compared to 20% coconut group (53.16 ± 3.86). They reported dose-dependent effect of coconut water in MDR percentages. In present study mean motility degradation rate (%) was significantly ($p<0.01$) lower in pre-freeze stage as compared to post-thaw stage and differed significantly ($p<0.01$) in all the groups. Atara *et al.* (2019) [34] reported increasing MDR with course of time at 30 (6.65 ± 0.24), 60 (13.67 ± 0.42), and 120 (28.77 ± 0.79) minutes of buck semen maintained at 37 °C. Contrary to the present findings Aguiar *et al.* (2013) [35] observed significantly ($p<0.05$) higher MDR at 2 hours after cooling (57.6 ± 9.1) as compared to 48 hours after cooling (43.5 ± 9.0) in dry season.

Conclusion

Addition of 4% Black cumin (*Nigella sativa*) seeds aqueous extract in tris egg yolk citrate extender maintained sperm motility above 65% at pre freeze and 45% at post thaw stage signify its favorable effect on cryopreservation of Surti buck semen.

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