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Original Research

Anti-Oxidant Effect of Quercetin in Tris Egg Yolk Citrate Extender on Surti Buck Semen Preserved at Refrigerated Temperature

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Abstract

A study was conducted to examine antioxidant effect of quercetin in tris egg yolk citrate extender on chilling quality of Surti buck semen. Total 72 semen ejaculates were collected from four Surti buck (18 ejaculate/buck) twice in a week by artificial vagina method. Semen samples were diluted with Tris egg yolk citrate extender and quercetin at different concentration viz. 0 µM/ml, 15 µM/ml, 25 µM/ml, 50 µM/ml, 75 µM/ml, 100 µM/ml and stored at refrigerated temperature (4-5 ºC). Evaluation of semen parameters was done at 0 and 48 hours. The result exhibited lower MDA level in 15 µM/ml quercetin added in tris egg yolk citrate extender group compared to control group in buck semen preserved at refrigerated temperature up to 48 hours. Marginally higher SOD activity was observed in 15 µM/ml quercetin group as compared to other treatment and control groups. Moreover, SOD values were observed to be decreasing with increase in quercetin concentration at both the time intervals.

Keywords: Antioxidant, Buck Semen, MDA, Quercetin, Refrigerated Temperature, SOD

Introduction

Livestock sector is an important subsector of the agriculture of Indian economy. Goat (*Capra hircus*) is one of the oldest domesticated species and one amongst the economically important livestock in India. Considering the poor production potential of goat, the need for genetic improvement via implementation of artificial insemination (AI) from superior sires through semen preservation is crucially required. Goat semen can be preserved either at room temperature temporarily, refrigerated temperature for 24 - 48 hours (Ferdinand *et al.*, 2012) or cryopreserved (Beltran *et al.*, 2013) for long term storage. During storage of mammalian spermatozoa, the phospholipids undergo peroxidation. The formation of toxic fatty acid peroxides causes structural damage to sperm cell accompanied by decreased motility (Jones and Mann, 1977; Mann *et al.*, 1980). Oxidative stress is a condition that is associated with an imbalance between the production and removal of reactive species and free radicals by antioxidants (Forman and Torres, 2002; Tremellen, 2008). Overproduction of ROS during cryopreservation/chilling creates an imbalance between ROS production and seminal plasma natural scavenger system which leads to sperm dysfunction (Mazzilli *et al.*, 1995) affecting semen characteristics. The protective capacity of endogenous antioxidants may be insufficient to prevent peroxide induced damage during storage (Aurich *et al.*, 1997). Therefore, the harmful action of free radicals can be blocked by exogenous antioxidant substances (Kumaran and Karunakaran, 2006). Natural most widely distributed dietary polyphenolic compounds antioxidant is quercetin, a non-enzymatic antioxidant (Nogueira *et al.*, 2013) belonging to aglycone flavonoid of the flavonols subclass (Kelly, 2011), found in plants. Quercetin has the ability to scavenge reactive species and hydroxyl radicals (Boots *et al.*, 2008) in the treatment of male infertility (Johinke *et al.*, 2014). Very meagre study has been conducted to test the antioxidant effects of quercetin in vitro in TRIS extender as Surti buck semen freezing medium. Thus, the present research work was conducted to elucidate the anti-oxidant effect of quercetin in tris egg yolk citrate extender on Surti buck semen preserved at refrigerated temperature.

Materials and Methods

Total of four apparently healthy Surti bucks about 1 years of age maintained under All India Coordinated Research Project (AICRP) on Goat at Livestock Research Station, Navsari Agricultural University, Navsari were selected. The selected bucks were housed in a common covered pen and managed under uniform managemental and feeding conditions. The animals were allowed to graze between 2:30 PM to 4:30 PM and fed with good quality fodder ad lib. along with 500 g of concentrate per animal per day. After completion of the training period, semen was collected twice a week from each buck by artificial vagina up to 9 weeks. Total 72 semen ejaculates were collected. Semen was collected from all the selected bucks twice weekly between 6.00 am to 8.00 am using eight-inch artificial vagina (AV) with 40 to 42 oC inner temperature and sufficient pressure.

Stock solution of Quercetin (1 mg/ml) was prepared and stored at refrigerated temperature till the day of use. Working solution (500 µM/ml) was prepared by adding 850 µl of MilliQ water to 150 µl of stock solution. Immediately after semen collection, the samples were pooled and only samples with > 70 % motility were considered for further processing. The pooled semen was divided into six aliquots and each aliquot was diluted with extender containing Tris-egg yolk citrate diluter with 0 µM/ml (control), 15 µM/ml (T1), 25 µM/ml (T2), 50 µM/ml (T3), 75 µM/ml (T4) and 100 µM/ml (T5) Quercetin was added to a final concentration of 200 x 106 sperm/ml. Lipid peroxidation in terms of Malonaldehyde (MDA) concentration were measured as per the method described by Rehman (1984) and superoxide dismutase (SOD) were evaluated at 0 and 48 hours as per Madesh and Balsubramanium (1998).

The data pertaining to various aspects were suitably tabulated and analysed using R-3.3.2 software. The differences among the parameter means were performed using appropriate statistical methods viz., ANOVA, DNMRT (Duncan's New Multiple Range Test). The mean differences were considered significant at $p<0.05$ and $p<0.01$.

Results and Discussion

Lipid Peroxidation (Malondialdehyde)

The initial (before preservation) and post-chilled MDA (nm/ml) concentration of ejaculated semen of Surti buck monitored in groups at different time intervals are presented in Table 1. The initial MDA level (nm/ml) at 0 hour was significantly ($p<0.01$) higher in control group as compared to T1 group and non-significantly higher when

compared to T2, T3, T4 and T5 groups. Post-chilled MDA level at 48 hours in control group was significantly ($p<0.01$) higher as compared to T1 group, however, it differed non-significantly between T2, T3, T4 and T5 groups. It was also observed that MDA level in all the groups increased with increasing preservation time. Moreover, MDA level was found in increasing trend with increased quercetin concentration.

Table 1: Effect of different concentrations of quercetin and storage duration on Malondialdehyde (nmol/ml) level of Surti buck semen preserved at refrigerated temperature (Mean ±SE)

Groups	MDA (nmol/ml) (n=18)		Overall (n=36)	t value	P value
	0 hr	48 hr			
C	$6.92\pm0.34^{ab}{}_{w}$	$7.93\pm0.37^{ab}{}_{w}$	7.42 ± 0.26^{bc}	4	0.05
T1	$5.55\pm0.20^{c}{}_{x}$	$6.56\pm0.41^{c}{}_{w}$	6.05 ± 0.24^{d}	4.98*	0.03
T2	$6.13\pm0.42^{bc}{}_{w}$	$7.11\pm0.58^{bc}{}_{w}$	6.62 ± 0.36^{cd}	1.8	0.18
T3	$7.07\pm0.51^{ab}{}_{w}$	$7.80\pm0.40^{ab}{}_{w}$	7.43 ± 0.33^{bc}	1.27	0.27
T4	$7.12\pm0.68^{ab}{}_{w}$	$8.02\pm0.33^{ab}{}_{w}$	7.57 ± 0.38^{ab}	1.45	0.24
T5	$8.02\pm0.39^{a}{}_{w}$	$8.80\pm0.35^{a}{}_{w}$	8.41 ± 0.27^{a}	2.19	0.15
Overall (n=108)	$6.80\pm0.19_{x}$	$7.70\pm0.18_{w}$	--	--	--
F value	3.66**	3.49**	--	--	--
P value	0	0	--	--	--

*$^{a-d}$Means with different superscript within a column (between the groups) differs significantly at $p<0.05$; $P<0.01$; $^{y-z}$Means with different subscript between a column (between time intervals) differs significantly at $p<0.05$; $p<0.01$; ** $p<0.01$; * $p<0.05$; C - Control; T1 - Quercetin 15µM/ml; T2 - Quercetin 25µM/ml; T3 - Quercetin 50µM/ml; T4 - Quercetin 75µM/ml; T5 - Quercetin 100µM/ml*

MDA level in T1 group compared to control, T2, T3, T4 and T5 groups were lower at different time intervals. In accordance to the above findings, Seifi-Jamadi *et al.* (2017) observed significantly ($p<0.001$) lower MDA (nm/mL) in quercetin 10µM group as compared to 20µM and control groups in freeze thawed goat semen. They concluded that supplementation of extender with 10 µM quercetin in combination with DMA suppressed lipid peroxidation by reducing the ROS formation and MDA concentration after freezing and thawing. Although, Avdatek *et al.* (2018) in his study found non-significantly lower MDA (nm/mL) in 25 µg/ml quercetin as compared to control group in post-thawed bull semen, it indicated that MDA activity was not affected positively by treatments. Moreover, they stated that ROS formation and membrane lipid peroxidation have negative effects on sperm parameters which are closely related to fertility.

Contrary to our result where 75 and 100 µM of quercetin was non-significantly higher than control at 48 hours post chilling, Ben Abdallah *et al.* (2011) reported beneficial effect of quercetin on LPO in rat epididymal spermatozoa. At 3 hours of incubation, they observed significantly ($p<0.05$) reduced level of MDA at higher concentrations (100 and 200 µM) of quercetin compared to control and 10 µM groups. Besides, Seifi-Jamadi *et al.* (2016) found no significant effect in MDA level at 0.1 mM of quercetin in post thawed stallion semen. Similarly, Khanduja *et al.* (2001) also found non-significant changes in human sperm MDA formation following 6 hours of incubation with 10µM, 50µM and 100µM quercetin as compared to control group.

In present study, MDA level was observed to have increased post-chilling at 48 hours compared to initial hour. Comparably, Khanduja *et al.* (2001) stated that gradual increase in MDA, in sperm fractions at 6 hours, has been attributed to oxidative stress to which spermatozoa are subjected during their in vitro storage. Similarly, Ghaniei *et al.* (2019) stated that amounts of MDA were increased significantly ($p<0.001$) in a time dependent manner in all groups at 0, 24 and 48 hours in rooster semen preserved at 4˚C. In-vivo administration of quercetin in Wistar rats significantly ($p<0.05$) decreased MDA level in the control and quercetin treated epididymal spermatozoa (Khaki *et al.*, 2010). Further, they also noted that oral feeding of quercetin for 28 days significantly improved epididymal sperm quality and numbers, and decreased the serum ROS. However, Yelumalai *et al.* (2019) in their in-vivo administration quercetin for 28 days found no significant changes in LPO level in the sperm of normal non-diabetic rats.

Quercetin is a flavonoid antioxidant, with beneficial effect on post thaw characteristics of buck sperm (Avdatek *et al.,* 2018). It was proposed that quercetin is able to reduce the amounts of MDA, prevent DNA damage and improve enzymatic antioxidant defence system when exposed to oxidative materials via scavenging free radicals and chelating divalent cations, which play important role in initiation of free radical reactions (Aherne and Brien, 2000; Ben Abdallah *et al.,* 2011).

Superoxide Dismutase (SOD) Level

The initial SOD (U/mg of protein) level before preservation and post-chilled SOD level in seminal plasma of ejaculated semen of Surti buck monitored in groups at different time intervals was presented in Table 2. The initial SOD level was non-significantly lower at 0 hour in control group, as compared to T1 and T2 groups and non-significantly higher when compared to T5 group. However, control differed non-significantly with T3 and T4 groups. Post-chilled SOD level at 48 hours, in control group was non-significantly lower as compared to T1, T2, T3, T4 and T5 groups. Furthermore, SOD level was found decreasing trend with increased quercetin concentration.

Table 2: Effect of different concentrations of quercetin and storage duration on Superoxide Dismutase (U/mg of protein) level of Surti buck semen preserved at refrigerated temperature (Mean ±SE)

Groups	SOD (U/mg of protein) (n=18)		Overall	t value	P value
	0 hr	48 hr	(n=36)		
C	$3.60\pm0.35^{ab}{}_{w}$	$3.31\pm0.32^{abc}{}_{w}$	3.46 ± 0.24^{bc}	0.35	0.56
T1	$4.45\pm0.34^{a}{}_{w}$	$4.20\pm0.39^{a}{}_{w}$	4.32 ± 0.26^{a}	0.23	0.63
T2	$4.23\pm0.28^{a}{}_{w}$	$3.68\pm0.40^{ab}{}_{w}$	3.95 ± 0.24^{ab}	1.25	0.27
T3	$3.85\pm0.23^{ab}{}_{w}$	$3.65\pm0.34^{ab}{}_{w}$	3.75 ± 0.20^{abc}	0.23	0.64
T4	$3.70\pm0.32^{ab}{}_{w}$	$2.75\pm0.39^{bc}{}_{w}$	3.22 ± 0.26^{cd}	3.6	0.06
T5	$2.94\pm0.24^{b}{}_{w}$	$2.39\pm0.28^{c}{}_{w}$	2.67 ± 0.19^{d}	2.17	0.15
Overall (n=108)	$3.79\pm0.13_{w}$	$3.33\pm0.15_{x}$	--	--	--
F value	3.12*	3.43**	--	--	--
P value	0.01	0	--	--	--

*$^{a-d}$ Means with different superscript within a column (between the groups) differs significantly at p<0.05; P<0.01; $^{x-w}$ Means with different subscript between a column (between time intervals) differs significantly at p<0.05; p<0.01; ** p<0.01; * p<0.05; C - Control; T1 - Quercetin 15µM/ml; T2 - Quercetin 25µM/ml; T3 - Quercetin 50µM/ml; T4 - Quercetin 75µM/ml; T5 - Quercetin 100µM/ml*

In context to superoxide dismutase levels, it was observed to be non-significantly higher at 15 µM quercetin group when compared to control group and significantly (p<0.01) higher than 100 µM quercetin group at 48 hours post freezing. The other hand, Ben Abdallah *et al.* (2011) observed that SOD activities were significantly increased in 100 and 200 µM quercetin group compared with control in three hours at 32˚C incubated rat epididymal spermatozoa. Ghaniei *et al*. (2019) detected that co-administration of H2O2 with 40 and 80 µM of quercetin in rooster semen, significantly (p<0.01) improved SOD activity in comparison with the control group at 48 hours of freezing, which was higher than 0 hour. But, in present finding SOD was non-significantly lower at 0 hour as compared to 24 hours. In vivo administration of quercetin in normal adult male rats by Yelumalai *et al.* (2019) found that SOD activity was high and did not significantly change following quercetin treatment.

Quercetin is an effective antioxidant because of not only its structural characteristics but also its ability to interact with and penetrate lipid bilayers. Therefore, the structure of quercetin plays an important role in attenuating the oxidative damage caused by hydrogen peroxide in reducing Lipid peroxidation and increasing antioxidant enzyme defence (Ben Abdallah *et al.,* 2011).

Conclusion

The study concluded that lower MDA level was observed in 15 µM/ml quercetin added in tris egg yolk citrate extender group compared to control group which indicate its suppressive effect on lipid peroxidation in buck semen preserved at refrigerated temperature up to 48 hours. Marginally higher SOD activity was observed in 15µM/ml

quercetin group as compared to other treatment and control groups indicate quercetin as an effective antioxidant may increase the sperm antioxidant defence in buck semen preserved at refrigerated temperature.

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Conflict of Interests

There is no conflict of interest.

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