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Research Article

PRESERVABILITY OF SURTI BUCK SEMEN IN TRIS EGG YOLK CITRATE EXTENDER ENRICHED WITH BEE'S HONEY

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ABSTRACT

A study was conducted to examine effects of bee honey supplementation into Tris egg yolk citrate extender on quality of Surti buck semen at refrigerated temperature. A total of 10 ejaculates were collected from the three bucks using an artificial vagina, once in a week from each buck up to 4 weeks. Semen samples were diluted with Tris–based extender (control) and Tris honey bee extender (1%, 2%, 3% and 4%). Both control and treated semen samples were preserved at refrigerated temperature (4-5 ºC) up to 48 hrs and evaluated semen parameters at every 24 hrs. The results revealed that addition of 3% and 4% honey in tris egg yolk citrate extenders maintained motility above 55% at 48 hours in Surti buck semen preserved at refrigerated temperature.

Keywords: Bee honey, Preservation, Semen, Surti buck

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Goat semen can be preserved either at room temperature temporarily, at refrigerated temperature for 24-48 hours (Ferdinand *et al.*, 2012) or cryopreserved (Beltran *et al.*, 2013) for long term storage. However, only limited period of storage at ambient temperature as well as poor quality on post-cryopreservation (Yimer *et al.*, 2014) encourage the workers to preserve the semen at refrigerated temperature which is cheaper and more feasible than cryopreservation of semen at ultra-low temperature. Moreover, by preserving semen at refrigerated temperature one can minimize the additional stress which occurs during cryopreservation of spermatozoa.

Honey is known to contain high amount of a variety of simple sugars (Fuller, 2004). Honey also contains trace amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Fakhrildin *et al.*, 2014). Some of the components were used solely as a source of energy in a semen extender (glucose, fructose and sucrose) (El-Sheshtawy *et al*., 2015), some as antioxidants (catalase, amino acids) (El-Sheshtawy *et al.*, 2013). Likewise, ascorbic acid was also tried by Sonmez and Demirci, 2004. Owing to the wide variety of properties of honey, its addition into a freezing medium is also expected to help spermatozoa protection during cryopreservation. Least studies have been conducted to test the effects of supplementing honey *in vitro* into tris extender as buck semen freezing medium.

MATERIALS AND METHODS

Sample collection and processing: Total three apparently healthy Surti male bucks above 2 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 managed under uniform managemental and feeding conditions. After completion of the training period, semen was collected regularly by artificial vagina once in a week from each buck up to 4 weeks. Total 10 semen ejaculates were collected from the selected bucks once in a week at early morning between 6.00 am to 8.00 am. The collected semen samples having >70% individual motility were selected for further analysis and extended with TEYC (Tris-2.42 gm; Sodium citrate- 1.36 gm; Fructose-1 gm; Streptomycin- 0.1gm; Penicillin- 1 lakh IU; Milli Q Water-100 ml; 20% egg yolk) diluter to make a final sperm concentration of 200×10^6 sperm/ml.

Preparation of Honey Extender : The product of Navsari Agricultural University, Navsari "Nauroji" Honey was used for the experiment. Honey solution was prepared by adding 1 ml honey to 9 ml distilled water (v:v) to obtain a honey solution of 10 % concentration and centrifuge the solution at 3000 rpm for 5 minute. After centrifugation, the supernatant was collected in sterile vial and stored at refrigerator temperature (4-5 ºC).

Experimental groups: To compare the effect of honey as additive on refrigerated semen parameters, 20 ml of diluted semen was divided into five aliquots and supplemented with 0% (control), 1% (T1), 2% (T2), 3% (T3) and 4% (T4) of honey extender to obtain a final volume of 5 ml in each tube.

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Components	Groups				
	Control	T1	T2	T3	T4
Diluted Semen	5 ml	4.5 ml	4.0 ml	3.5 ml	3 ml
10% Honey Extender	-	0.5 ml	1.0 ml	1.5 ml	2.0 ml
Total volume	5 ml	5 ml	5 ml	5 ml	5 ml

Semen evaluation: The parameters studied were subjective semen characteristics, viz., (individual motility (%), live-dead sperm count (%), morphological abnormality (%), HOST reacted sperms (%), motility degeneration rate (%)) immediately after the dilution with standard method and considered at 0 hrs. Further, the semen samples were preserved at refrigerated temperature (4-5 ºC) up to 48 hrs and evaluated semen parameters at every 24 hours interval.

Statistical analysis: 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

Initial Motility: The initial motility was similar in all treatment and control groups at 0 hour. Post-chilled individual sperm motility (%) at 24 hours was significantly ($p<0.01$) lower in control group as compared to T2, T3 and T4 groups, whereas, it was non-significantly differed among T1, T2, T3 and T2, T3, T4 groups. Post-chilled individual sperm motility (%) at 48 hours was significantly ($p<0.01$) lower in control group as compared to T1, T2, T3 and T4 groups, whereas, it was similar in T1, T2 and T3 as well as T3 and T4 groups. The corresponding pooled mean of sperm motility irrespective of treatment and control groups were reduced with increasing preservation time at 0 (80.00±2.45) hour followed by 24 (66.20±2.67) and 48 (52.60±2.85) hours (Table.1).

Moreover, in all the groups, individual sperm motility was significantly ($p<0.01$) higher at 0 hour as compared to 24 and 48 hours. The corresponding pooled mean of sperm motility irrespective of time interval was lower in control group as compared to T1, T2, T3 and T4 groups (Table 1). In accordance to the present findings, Olayemi *et al.* (2011) in buck; Banday *et al.* (2017), Zaghloul (2017) in ram reported higher motile sperm in honey additives groups as compared to control group in their experiments.

In present study, highest post chilled individual motility was found in T4 group (4% honey), which is in agreement with Zaghloul (2017) who found higher post chilled sperm motility at 48 hours in Tris based extender with 3 or 4.5% honey than the control and other groups in Barky ram semen. Whereas, contrary to the present findings, Olayemi *et al.* (2011) observed highest percentage motility at 2, 4 and 6 hours post chilled in 5% honey extender in buck semen.

Live sperm count: The live sperm count (%) at 0 and 24 hour differed non-significantly between control, T1, T2, T3 and T4 groups. Post-chilled live sperm count (%) at 48 hour was non-significantly lower in control group as compared to T1 and T2 groups and significantly ($p<0.05$) lower as compared to T3 and T4 groups. However, the difference between T1, T2, T3 and T4 were non-significant in present experiment. The corresponding pooled mean of live sperm count (%) irrespective of treatment and control groups were reduced with increasing preservation time at 0

Table 1

Effect of different concentrations of tris honey extender and storage duration on motility (%) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	Motility (%) (n=10)			Overall	F value	P value
	0 hr	24 hr	48 hr			
C	80.00±2.50^{ax}	59.50±2.76^{cy}	43.50±2.50^{cz}	61.00±4.07	73.78**	0.00
T1	80.00±2.50^{ax}	64.50±2.54^{bcy}	50.00±2.40^{bz}	64.80±3.71	59.44**	0.00
T2	80.00±2.50^{ax}	66.50±2.30^{aby}	53.50±2.41^{bz}	66.70±3.51	52.38**	0.00
T3	80.00±2.50^{ax}	69.00±2.38^{aby}	55.50±2.45^{abz}	68.20±3.42	42.24**	0.00
T4	80.00±2.50^{ax}	71.50±2.18^{ay}	60.50±2.45^{az}	70.70±3.13^{a}	29.49**	0.00
Overall	80.00±2.45	66.20±2.67	52.60±2.85	—	—	—
F value	0	5.75**	11.33**	—	—	—
P value	1	0.00	0.00	—	—	—

$^{a-c}$Means with different superscript within a column (between the groups) differs significantly at $p<0.05$; $p<0.01$; $^{x-z}$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- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%

(81.00±2.41) hour followed by 24 (75.90±2.79) and 48 (72.90±2.98) hours (Table 2).

Moreover, in control, T1 and T2 groups, live sperm counts (%) were non-significantly higher at 0 hour as compared to 24 hours and significantly ($p<0.05$) higher as compared to 48 hours, however, the difference between 24 and 48 hours was non-significant. In T3 and T4 group, live sperm count (%) was non-significantly higher at 0 hour as compared to 24 and 48 hours. The corresponding pooled mean of live sperm count (%) irrespective of time interval was lower in control group followed by T1, T2, T3 and T4 groups (Table 2). In accordance to the present findings, Olayemi *et al.* (2011) in buck and Banday *et al.* (2017) in ram reported higher live sperm count (%) in honey additives groups as compared to control group in their experiments.

In present study, highest post chilled live sperm count (%) was found in 3-4 % honey added group at 48 hours, which is in agreement with Zaghloul (2017) who found higher post chilled live sperm count (%) at 48 hours in Tris based extender with 4.5% honey than the control and other groups in Barky ram semen. Whereas, contrary to the present findings, Olayemi *et al.* (2011) observed highest liveability percentage of sperm at 2, 4 and 6 hours post chilled in 5% honey extender in buck semen. Banday *et al.* (2017) observed post-thaw live sperm count for T1 (2.5% honey) significantly ($p<0.05$) lower than control but significantly ($p<0.05$) higher than T2 (5% honey) and T3 (7% honey) treatment groups in crossbred ram semen. The liveability of cooled extended buck spermatozoa displayed was non-significantly higher in honey added groups as compared to control group. Natural honey contains not only sugars (glucose, fructose and sucrose), but also vitamins and amino acids which have their impact on liveability of spermatozoa (Bamba *et al.*, 1968).

HOST reacted sperm count: The HOST reacted sperm count (%) was similar in all treatment and control groups at 0 hour. Post-chilled HOST reacted sperm count (%) at 24 hours was significantly ($p<0.05$) lower in control group as compared to T4 group. Post-chilled HOST reacted sperm count (%) at 48 hour was significantly ($p<0.05$) lower in control group as compared to T3 and T4 groups. The corresponding pooled mean of HOST reacted sperm count (%) irrespective of treatment and control groups were reduced with increasing preservation time at 0 hour followed by 24 and 48 hours (Table 3).

Moreover, in control, T1, T3 and T4 groups, HOST reacted sperm count (%) were non-significantly higher at 0 hour as compared to 24 hours and significantly ($p<0.01$) higher as compared to 48 hours. In T2 group, HOST reacted sperm count (%) differed non-significantly between 0, 24 and 48 hours. The corresponding pooled mean of HOST reacted sperm count (%) irrespective of time interval was lower in control group as compared to T1, T2, T3 and T4 groups (Table 3). Contrary to the present findings, Banday *et al.* (2017) found post-thaw HOST reacted sperm count (%) significantly higher ($p<0.05$) in control (60.33 ± 1.42) than all the other Tris-based honey extender groups in ram semen.

Abnormal sperm count (%): The abnormal sperm count (%) at 0, 24 and 48 hours differed non-significantly between control, T1, T2, T3 and T4 groups in present experiment. The corresponding pooled mean of abnormal sperm count (%) irrespective of treatment and control groups were increased with increasing preservation time at 0 hour followed by 24 and 48 hours (Table 4).

Moreover, in all the groups, abnormal sperm count

Table 2

Effect of different concentrations of tris honey extender and storage duration on live sperm count (%) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	Live sperm count (%) (n=10)			Overall	F value	P value
	0 hr	24 hr	48 hr			
C	77.90±2.45^{x}	71.60±2.95^{xy}	66.10±3.06^{by}	71.90±3.05	5.22*	0.02
T1	79.80±2.35^{x}	74.60±2.60^{xy}	72.10±2.88^{aby}	75.50±2.73	3.21*	0.05
T2	80.80±2.47^{x}	75.90±2.78^{xy}	72.80±2.86^{aby}	76.50±2.81	2.97*	0.05
T3	83.00±2.28^{x}	78.20±2.70^{xy}	76.40±2.77^{axy}	79.20±2.67	2.51	0.10
T4	83.60±2.34^{x}	79.10±2.71^{x}	77.30±2.77^{ax}	80.00±2.68	2.21	0.12
Overall	81.00±2.41	75.90±2.79	72.90±2.98	—	—	—
F value	1.70	1.55	2.87*	—	—	—
P value	0.17	0.20	0.03	—	—	—

$^{a-b}$Means with different superscript within a column (between the groups) differs significantly at $p<0.05$; $p<0.01$. $^{x-y}$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- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%.

Table 3

Effect of different concentrations of tris honey extender and storage duration on HOST reacted-spermatozoa (%) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	HOST reacted- spermatozoa (%) (n=10)			Overall	F value	P value
	0 hr	24 hr	48 hr			
C	63.50±2.84^{x}	57.70±2.62^{bxy}	52.30±2.57^{by}	57.80±2.89	6.04**	0.01
T1	69.50±2.99^{x}	63.00±2.80^{abxy}	58.60±2.62^{aby}	63.70±2.98	4.77*	0.02
T2	66.70±3.11^{x}	62.50±2.90^{abx}	59.10±2.61^{abx}	62.80±2.95	2.06	0.15
T3	70.00±2.97^{x}	64.70±2.80^{abxy}	61.80±2.91^{ay}	65.50±2.97	3.68*	0.04
T4	71.60±2.41^{x}	68.20±2.61^{axy}	62.50±2.65^{ay}	67.40±2.72	4.90*	0.02
Overall	68.30±2.92	63.20±2.84	58.90±2.79	—	—	—
F value	1.46	2.52*	3.15*	—	—	—
P value	0.23	0.05	0.02	—	—	—

$^{a-b}$Means with different superscript within a column (between the groups) differs significantly at p<0.05; p<0.01. $^{x-y}$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- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%

Table 4

Effect of different concentrations of tris honey extender and storage duration on abnormal sperm count (%) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	Abnormal sperm count (%) (n=10)			Overall	F value	P value
	0 hr	24 hr	48 hr			
C	4.00±1.33^{z}	6.80±1.47^{y}	9.60±1.54^{x}	6.80±1.76	17.64**	0.00
T1	4.00±1.30^{z}	6.60±1.69^{y}	9.10±1.75^{x}	6.60±1.81	9.58**	0.00
T2	3.30±1.30^{y}	6.30±1.55^{x}	8.40±1.65^{x}	6.00±1.76	11.94**	0.00
T3	3.30±1.35^{z}	5.60±1.46^{y}	8.30±1.50^{x}	5.70±1.69	15.02**	0.00
T4	3.30±1.25^{z}	6.00±1.28^{y}	8.60±1.55^{x}	6.00±1.69	19.25**	0.00
Overall	3.60±1.30	6.30±1.49	8.80±1.58	—	—	—
F value	0.50	0.44	0.45	—	—	—
P value	0.74	0.78	0.77	—	—	—

$^{a-b}$Means with different superscript within a column (between the groups) differs significantly at p<0.05; p<0.01. $^{x-z}$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- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%

(%) were significantly (p<0.01) lower at 0 hour as compared to 24 and 48 hours. The corresponding pooled mean of abnormal sperm count (%) irrespective of time interval was higher in control group as compared to T1, T2, T3 and T4 groups (Table 4).

In present study, non-significant lower post chilled abnormal sperm count (%) was found in T3 group (3 % honey) at 48 hours as compared to control, which is in agreement with Zaghloul (2017) who observed that mean percentage of abnormal spermatozoa of ram semen was significantly (p<0.05) lower in groups supplemented with HB 3.0 and HB 4.5 ml compared to control and HB 1.5 ml at 0, 24 and 48 hours after chilled storage.

Motility degeneration rate (%): The motility degeneration rate (%) at 24 hours was significantly (p<0.01) higher in control group as compared to T1, T2, T3 and T4 groups. The motility degeneration rate (%) was significantly lower in T4 group as compared to control, T1, T2 and T3 groups. The motility degeneration rate (%) at 48 hour was significantly (p<0.01) higher in control group as compared to T1, T2, T3 and T4 groups, The corresponding pooled mean of motility degeneration rate (%) irrespective of treatment and control groups were increased with increasing preservation time at 24 (17.00±2.53) hours followed by 48 (34.10±3.15) hours (Table 5).

In accordance to the present study, Atara *et al.* (2018) also found increase motility degeneration rate with increasing preservation time at 30 min, 60 min and 120 min. Whereas, opposite to the present findings, Lima *et al.* (2013) and Aguiar *et al.* (2013) observed no such type of increasing trend.

Table 5

Effect of different concentrations of tris honey extender and storage duration on percent motility degeneration rate (MDR) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	MDR (%) (n=10)				
	24hr	48 hr	Overall	F value	P value
C	25.15±2.43^{az}	45.61±2.56^{ay}	35.38±3.48	53.81**	0.00
T1	19.45±1.86^{bz}	37.35±2.63^{by}	28.40±3.26	53.48**	0.00
T2	16.81±1.90^{bcz}	32.96±2.63^{bcy}	24.88±3.14	42.85**	0.00
T3	13.75±1.50^{cz}	30.44±2.71^{cdy}	22.09±3.17	47.19**	0.00
T4	9.89±1.68^{dz}	24.15±2.74^{dy}	17.02±3.03	31.47**	0.00
Overall	17.00±2.53	34.10±3.15	—	—	—
F value	22.80**	12.88**	—	—	—
P value	0.00	0.00	—	—	—

$^{a-d}$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- Tris Honey 1%; T2-Tris Honey 2%; T3-Tris Honey 3%; T4-Tris Honey 4%

CONCLUSIONS

Addition of 3 % and 4 % honey in tris egg yolk citrate extenders maintained motility above 55% at 48 hours in Surti buck semen preserved at refrigerated temperature which proves its beneficial effect on semen preservation. However, routine use of such addition in goat semen extender can be recommended after performing field fertility trials.

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