RESEARCH ARTICLE

Effect of Different Egg Yolk Levels in Tris Extender With and Without Aloe Vera and Onion Crude Extracts on Surti Buck Semen at Refrigerated Temperature

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Abstract

This study aimed to develop a suitable Tris-citrate-fructose-egg yolk (Tris) based extender with Aloe vera or onion extract to preserve Surti buck semen at refrigerated temperature. Semen ejaculates (n=96) collected by artificial vagina from eight bucks were split-diluted in Tris extender with 0, 5, 10, 20% egg yolk concentrations. All these were then fractionated into three aliquiots each, one fracrtion kept without further additives (EY0, EY5, EY10, EY20), while fractions 2 and 3 were an addition with crude filtrates of Aloe vera gel @ 10 µL (EY0+A, EY5+A, EY10+A, EY20+A) or onion extract @ 10 µL (EY0+O, EY5+O, EY10+O, EY20+O) per 0.5 ml of the extended semen. These samples (4x3 = 12 aliquots, each 0.5 ml) were then preserved at refrigerated temperature and evaluated at 12 hourly interval up to 48 h of storage for various sperm quality tests. Sperm motility was significantly lower ($p<0.01$) in all the subgroups with 0% egg yolk (EY0, EY0+A, EY0+O) compared to 5 to 20% egg yolk at all intervals. The sperm motility at 48 h was significantly ($p<0.01$) lower in EY0 without and with Aloe vera and onion (11.49 ± 2.54, 10.12 ± 2.45 and 6.58 ± 2.30 %) than EY5 without and with Aloe vera and Onion extract (53.85 ± 1.41, 56.82 ± 1.20, 34.84 ± 2.25%), EY10 without and with Aloe vera and onion (55.83 ± 1.19, 57.76 ± 1.10, 35.37 ± 2.15%), EY20 without and with Aloe vera and onion extract (56.09 ± 1.32, 56.46 ± 1.26 and 41.06 ± 1.92%, respectively). The live sperm count was significantly lower ($p<0.01$) in semen extended with 0% egg yolk as compared to 5, 10 and 20% egg yolk at 0, 24 and 48 hours. The post-chilled abnormal sperm percentage increased non-significantly at 24 and 48 hours in all extender combinations, although the effect of additives was non significant. It is concluded that adding egg yolk alone 5–20% or with Aloe vera extract @ 10 µL in semen extender had a beneficial role in Surti buck semen preservation at refrigerated temperature.

Keywords: Aloe vera, Egg yolk, Onion, Semen quality, Surti buck

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Introduction

Goats play an important role in the food and nutritional security of the rural poor especially in the rain-fed regions where crop production is uncertain, and rearing large ruminants is restricted by acute scarcity of feed and fodder (Kumar *et al*., 2010). Looking to the poor production potential of goat and at the same time importance of its milk, genetic improvement via implementation of Artificial Insemination (AI) programme through semen preservation is crucially required. This is possible only with semen preservation of superior bucks with suitable extenders for an extended period, as it is essential for the success of AI programme. Goat semen can be preserved either at room temperature, at refrigerated temperature for 24–48 h or cryopreserved for long-term storage. Preservation of goat semen at refrigerated temperature is cheaper and more feasible than cryopreservation of semen at ultra-low temperature (Yimer *et al*., 2014). To preserve goat sperm, different egg yolk concentrations (2.5 to 20%) were compared by different workers (Ranjan *et al*., 2009; Priyadharsini *et al*., 2011; Kalyani *et al*., 2015; Gojen Singh *et al*., 2016).

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Free radicals and reactive oxygen species (ROS), being associated with oxidative stress; play a pivotal role in reproduction (Adewoyin *et al*., 2017). Significant structural alterations occur even in the presence of cryoprotectants such as glycerol and egg yolk. Exudates of *Aloe barbadensis* leaves possess antioxidant activity shown by increased superoxide dismutase (SOD) scavenging activity and decreased lipid peroxidation in rats (Nwajo, 2006). Aloe vera has strong spermatogenic activity by increasing sperm parameters, *viz.,* epididymal sperm counts and sperm motility. It can be a good candidate for manufacturing fertility drugs (Estakhr and Javdan, 2011). Onion bulbs (*Allium cepa* L.) are among the richest sources of dietary flavonoids and contribute to a large extent to the overall intake of flavonoids (Slimestad *et al*., 2007). Flavonoids are potent antioxidants, free radical scavengers, and metal chelators and inhibit lipid peroxidation (Cook and Samman, 1996). Thus, the present study was designed to develop suitable extender to preserve Surti buck semen at refrigerated temperature using natural antioxidants, *viz.,* Aloe vera and onion crude extract, and egg yolk.

MATERIALS AND METHODS

The study was carried out on eight apparently healthy Surti male bucks above 1 ½ years of age maintained under All India Coordinated Research Project (AICRP) on Goat at Livestock Research Station, Navsari Agricultural University, Navsari. The selected bucks were housed in a common covered pen and separated from females.

Preparation of Aloe Vera and Onion Crude Filtrate:

On the previous day of the semen collection, fresh Aloe vera leaves were collected and washed under running tape water. After drying the leave with clean dry cloth, the spines were removed by a sharp knife. The lower and upper thin layers were scraped off to expose the gel core. The small pieces of Aloe vera gel core were collected in a sterile petri dish. Similarly, fresh onion bulbs were obtained from the market. After removing the outer part, the bulbs were chopped into smaller pieces and minced in the juicer. The Aloe vera and onion crude mixtures were then extracted by mincing the portions in a juicer. The extracted crude mixtures were poured into sterile centrifuge tubes and centrifuged at least for 4 min at 670 x g. After centrifugation, the supernatants were carefully aspired in 5 mL sterile syringes and filtered with 0.42 µM syringe filter to get the clear filtrate of Aloe vera and onion into microcentrifuge tubes. The extracts were stored in the refrigerator at 5°C till use.

Preparation of Diluents:

The Tris-citrate-fructose-yolk diluents with different egg yolk concentrations (0, 5, 10 and 20%) were prepared on the previous day of the semen collection. The mixtures were thoroughly mixed with magnetic stirrer in the flasks for five minutes, poured into sterile centrifuge tubes and centrifuged for 4 min at 670 x g. After centrifugation, the supernatants from each tube were carefully obtained into sterile glass bottles and stored in the refrigerator at 5°C until use on the next day.

Semen Collection:

The bucks were trained to donate the semen in artificial vagina using male as dummy. After completion of the training period, semen was collected regularly by the artificial vagina once in a week from each buck up to 12 weeks. During the study period, 96 semen ejaculates (12 from each of 8 bucks) were collected.

Experimental Design and Groups:

Immediately after evaluation, each fresh semen sample was divided in to four parts (0.1 mL each) and extended with 1.4 mL extender containing different egg yolk concentrations (0, 5, 10 and 20%). After dilution, the semen samples were examined (0 h) for initial motility, live sperm count and abnormal sperm count as contro. To compare the effect of Aloe vera and onion crude filtrates as additive on refrigerated semen parameters, all the above split-semen samples extended with different egg yolk concentrations were divided in to three identical aliquots (total 4x3=12 aliquots, 0.5 ml each). The first and second aliquots were supplemented with 10 µL Aloe vera extract (EY0+A, EY5+A, EY10+A and EY20+A) or 10 µL Onion extract (EY0+O, EY5+O, EY10+O and EY20+O) as natural antioxidants while remaining third aliquots (EY0, EY5, EY10 and EY20) of the same samples were kept as unadded controls. All the above 12 tubes with extended semen in Tris at different concentrations of egg yolk without or with herbal extracts were covered with aluminium foil and preserved at refrigerated temperature (5°C) and evaluated periodically up to 48 h for individual sperm motility (12, 24, 36 and 48 h), and live sperm count and sperm morphology (24 and 48 h).

Statistical Analysis:

The data was analyzed by one-way ANOVA using SPSS (Statistical Package for Social Sciences, Version 20.0) software. Descriptive statistics specifying Mean ± SE were calculated for each group. The means were compared by Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

The initial motility differed non-significantly at 0 h between EY0, EY5, EY10 and EY20 groups (Table 1), which was in accordance with Kumar *et al.* (2018). Post-chilled individual sperm motility (%) at 12, 24, 36 and 48 h were significantly ($p<0.01$) lower in all the three subgroups without or with Aloe vera and onion at 0% egg yolk (EY0, EY0+A and EY0+O) than all the other groups at 5, 10 and 20% egg yolk. Moreover, post-chilled individual sperm motility was consistently higher at all intervals at all the higher concentrations of egg yolk (5–20%) with Aloe vera compared to their counterparts, *viz.,* without

Table 1: Percent sperm motility of Surti buck semen (Mean ± SE) at refrigerated temperature in different extender-additives

Groups	Additives	Sperm motility (%)				
		0 h	12 h	24 h	36 h	48 h
	EY0	80.26 ± 0.46^{a} (96)	42.03 ± 2.27^{b} (96)	18.76 ± 1.83^{b} (89)	11.69 ± 1.88^{b} (65)	11.49 ± 2.54^{a} (37)
0% EY (EY0)	EY0+A	81.05 ± 0.38^{a} (96)	42.86 ± 2.25^{b} (96)	21.40 ± 2.06^{b} (86)	12.82 ± 1.91^{b} (62)	10.12 ± 2.45^{a} (41)
	EY0+O	78.19 ± 0.66^{b} (96)	33.07 ± 2.14^{a} (96)	12.91 ± 1.55^{a} (79)	6.94 ± 1.72^{a} (54)	6.58 ± 2.30^{a} (19)
	EY5	80.73 ± 0.48^{a} (96)	74.38 ± 0.53^{cd} (96)	68.59 ± 0.73^{d} (96)	62.29 ± 1.07^{d} (96)	53.85 ± 1.41^{d} (96)
5% EY (EY5)	EY5+A	81.46 ± 0.48^{a} (96)	75.05 ± 0.57^{cd} (96)	69.32 ± 0.83^{d} (96)	64.22 ± 0.95^{d} (96)	56.82 ± 1.20^{d} (96)
	EY5+O	77.97 ± 0.59^{b} (96)	71.51 ± 0.71^{c} (96)	60.10 ± 1.38^{c} (96)	50.83 ± 1.88^{c} (96)	34.84 ± 2.25^{b} (93)
	EY10	80.73 ± 0.48^{a} (96)	75.00 ± 0.57^{cd} (96)	68.96 ± 0.72^{d} (96)	63.33 ± 0.85^{d} (96)	55.83 ± 1.19^{d} (96)
10% EY (EY10)	EY10+A	81.27 ± 0.43^{a} (96)	75.42 ± 0.52^{cd} (96)	69.58 ± 0.74^{d} (96)	64.48 ± 0.86^{d} (96)	57.76 ± 1.10^{d} (96)
	EY10+O	78.89 ± 0.57^{b} (96)	72.08 ± 0.84^{cd} (96)	61.46 ± 1.50^{c} (96)	53.32 ± 1.59^{cd} (95)	35.37 ± 2.15^{b} (94)
	EY20	80.73 ± 0.48^{a} (96)	74.95 ± 0.56^{cd} (96)	69.11 ± 0.72^{d} (96)	64.11 ± 0.84^{d} (96)	56.09 ± 1.32^{d} (96)
20% EY (EY20)	EY20+A	81.92 ± 0.37^{a} (96)	75.83 ± 0.60^{d} (96)	69.64 ± 0.69^{d} (96)	65.00 ± 0.81^{d} (96)	56.46 ± 1.26^{d} (96)
	EY20+O	78.74 ± 0.62^{b} (96)	71.77 ± 0.92^{c} (96)	62.76 ± 1.45^{c} (96)	55.68 ± 1.48^{d} (95)	41.06 ± 1.92^{c} (94)
F value		8.67*	162.48**	305.13**	237.29**	81.46**
P value		0.03	0.00	0.00	0.00	0.00

$^{a-d}$Means with different superscript within a column differs significantly at p<0.05.

NS=Non-significant; **Highly significant (p<0.01), Figures in parenthesis indicate number of sample

any additive or with onion. The later suppressed the sperm motility in all extender combinations at all chilled-storage intervals, and it must have depressed the sperm motility soon after addition because of its astringent and irritant properties. However, we did not record it (Table 1). Similarly, sperm motility was highest at 0% egg yolk with Aloe vera compared to their counterparts at all the periods, except at 48 h. This effect on sperm motility may be due to the antioxidant component of Aloe vera. The findings indicated the beneficial role of Aloe vera as natural antioxidant in improving the keeping quality of goat semen preserved at refrigerator temperature. Like present findings, many previous researchers observed higher progressive motile sperm in natural additives (honey, soybean lecithin, crude fig fruit filtrate, coconut water, orange/cucumber/ pineapple juices, avocado seed extract) than those of control (Olayemi *et al.,* 2011; Khalifa and Abdel-Hafez, 2013; Zaenuri *et al*., 2014; Daramola *et al.,* 2016[a,b]). Estakhr and Javdan (2011) also found significantly higher sperm motility in mice treated with Aloe vera as compared to control group. However, contrary to the present findings, lower progressive motility after Aloe vera treatment in West African Dwarf bucks has been observed (Olugbenga *et al.,* 2011).

Semen samples with below 5% motility were excluded from the statistical analysis. In the present experiment, semen samples without egg yolk (EY0) showed significantly (p<0.01) lower sperm motility compared to the samples with different concentrations of egg yolk viz., EY5, EY10 and EY20 at all storage intervals beyond 12 hours. The above findings indicate the importance of egg yolk in semen dilutor to improve the quality of goat semen preserved at refrigerator temperature. Further, the motility differed non-significantly between all egg yolk concentrations with gradual reduction from 20 to 0% egg yolk till 48 h of storage. Moreover, the values in Aloe vera added extender and control one were statistically similar and higher than in onion added extender at almost all periods, and 24 h onward in particular, of chilled preservation. A similar trend of significantly higher motility

Table 2: Live sperm count (%) of Surti buck semen (Mean ± SE) at refrigerated temperature in different extender-additives=96

Groups Additives		Live sperm count (%)		
		0 h	24 h	48 h
0% EY (EY0)	EY0	88.45 ± 0.49a	41.84 ± 2.45b	19.02 ± 1.83b
	EY0+A	--	40.29 ± 2.42b	19.02 ± 1.83b
	EY0+O	--	27.81 ± 2.02a	11.03 ± 1.29a
5% EY (EY5)	EY5	91.99 ± 0.37b	86.31 ± 0.63cdef	78.34 ± 1.13e
	EY5+A	--	87.20 ± 0.80def	80.14 ± 1.20ef
	EY5+O	--	82.73 ± 0.94c	67.89 ± 1.93c
10% EY (EY10)	EY10	92.44 ± 0.37bc	86.81 ± 0.65cdef	80.57 ± 1.03ef
	EY10+A	--	87.68 ± 0.70def	81.47 ± 1.18ef
	EY10+O	--	83.58 ± 0.86cd	68.76 ± 1.96cd
20% EY (EY20)	EY20	93.19 ± 0.38c	88.17 ± 0.59ef	81.37 ± 1.20ef
	EY20+A	--	89.23 ± 0.59f	83.27 ± 1.11f
	EY20+O	--	84.95 ± 0.83cde	72.36 ± 1.68d
F value		26.79**	297.73**	354.90**
P value		0.00	0.00	0.00

a-fMeans with different superscript within a column differs significantly at $p<0.05$; ** $p<0.01$.

Table 3: Abnormal sperm count (%) of Surti buck semen (Mean ± SE) at a refrigerated temperature in different extender-additivesn=96

Groups Additives		Abnormal sperm count (%)		
		0 h	24 h	48 h
0% EY (EY0)	EY0	5.58 ± 0.18	7.75 ± 0.29	9.30 ± 0.34
	EY0+A	--	7.68 ± 0.28	9.20 ± 0.33
	EY0+O	--	7.85 ± 0.25	9.55 ± 0.32
5% EY (EY5)	EY5	5.27 ± 0.16	7.02 ± 0.24	8.69 ± 0.29
	EY5+A	--	7.10 ± 0.23	8.59 ± 0.27
	EY5+O	--	7.56 ± 0.25	9.32 ± 0.32
10% EY (EY10)	EY10	5.29 ± 0.16	7.13 ± 0.23	8.74 ± 0.28
	EY10+A	--	7.15 ± 0.22	8.91 ± 0.30
	EY10+O	--	7.58 ± 0.25	9.22 ± 0.31
20% EY (EY20)	EY20	5.53 ± 0.18	7.26 ± 0.23	8.67 ± 0.28
	EY20+A	--	7.35 ± 0.24	8.85 ± 0.30
	EY20+O	--	7.71 ± 0.24	9.47 ± 0.31
F value		0.89 NS	1.41 NS	1.23 NS
P value		0.45	0.16	0.26

A=Aloe vera, O=Onion, NS=Non-significant

at higher egg yolk concentrations than lower one has been observed in the literature (Ranjan *et al*,. 2009; Anand *et al.,* 2016). However, contrary to our findings, significantly higher motility at lower egg yolk concentrations has been recorded in some other studies (Kalyani *et al.,* 2015; Gojen Singh *et al.,* 2016). The low-density lipoproteins (LDLs) of egg yolk though protect the sperm against damage during the storage, cooling and freezing process (Vidal *et al.,* 2013), the increased hydrolysqis of lecithin with increased egg yolk level in diluted semen leads to deterioration of sperm motility (Kalyani *et al.,* 2015).

In present experiment, live sperm count at 0 h was significantly ($p<0.01$) higher in EY5, EY10 and EY20 groups than EY0 (Table 2). In contrast to the present report, Ranjan *et al.* (2009) reported a non-significant difference in live sperm count at 0 h with 2.5, 5.0, 7.5 and 10% egg yolk level. Post-

chilled live sperm count (%) at 24 and 48 h was significantly ($p<0.01$) lower in EY0+O than other groups. Moreover, significantly lower ($p<0.01$) live sperm count was observed in all the three subgroups without and with addiives at 0% egg yolk (EY0, EY0+A and EY0+O) than all the other groups at 5, 10 and 20% egg yolk at all storage (24 and 48 h) intervals. Similar trend of higher live sperm count at higher egg yolk concentrations has been reported by Ranjan *et al.* (2009) and Anand *et al*. (2016). However, others (Kalyani *et al*., 2015; Gojen Singh *et al*., 2016) reported a significantly inverse trend of higher live sperm count at lower egg yolk concentration. Post-chilled live sperm count was consistently higher with Aloe vera in all the egg yolk concentrations (5–20%) as compared to their counterparts, *viz.,* without additive or with onion at 24 and 48 h. In concurrence with the present findings, some workers (Olayemi *et al.,* 2011; Khalifa and Abdel-Hafez, 2013; Zaenuri *et al*., 2014) also recorded higher live sperm count in natural additive groups as compared to control group. However, lower live sperm count after Aloe vera gel treatment in West African Dwarf bucks has been reported (Olugbenga *et al.,* 2011).

The abnormal sperm count, though increased gradually from 0, 24 to 48 h of storage, did not differ significantly at any interval between groups (Table 3). Moreover, post-chilled abnormal sperm count was consistently higher at all the concentrations of egg yolk (5-20%) with onion (EY5+O, EY10+O, EY20+O) followed by Aloe vera (EY5+A, EY10+A, EY20+A) at both 24 and 48 h of storage in present experiment, while the abnormalities were lowest without any additive (EY5, EY10, EY20) groups. Similarly, higher abnormal sperm percentage after Aloe vera treatment in West African Dwarf bucks (Olugbenga *et al.,* 2011), and the non-significant difference between different groups (Daramola *et al.,* 2016a) has been documented, while contradictory findings were reported in other studies (Khalifa and Abdel-Hafez, 2013; Daramola *et al.,* 2016b).

Non-significantly higher sperm abnormalities were observed in EY0 than EY5, EY10 and EY20 groups in the present study. Similar higher sperm abnormalities at higher egg yolk have been reported by other researchers also (Kalyani *et al.,* 2015; Gojen Singh *et al*., 2016).

CONCLUSION

The findings showed that the acceptable quality of Surti buck semen in terms of sperm motility, and liveability can be preserved in Tris-citrate-fructose-egg yolk extender at refrigeration temperature (5° C) up to 48 h. Addition of varying levels of egg yolk (0, 5, 10, 20%) alone or with Aloe vera natural gel @ 10 µL per 0.5 mL of semen extender had a beneficial role in Surti buck semen preservation under refrigerated temperature. At the same time, onion extract has a detrimental effect on sperm motility and viability.

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