Upshot of washing of Prepuce on bacterial load of semen and its consequences on semen quality in Sahiwal bulls

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Abstract

Douching and cleaning of preputial cavity of bulls used for semen collection is very important for quality semen production. Preputial washing reduces the semen microbial contamination to a greater extent without harmful effect on semen quality and preservability. By effective douching and cleaning of prepuce, based on measured capacity of prepuce is of prime importance for quality semen production. It is well established that prepucial capacity may vary from breed to breed and, hence, for optimum douching and cleaning of prepuce of a bull, volume used for douching, may also vary. Hence, keeping in views of the fact the present works was conducted with aim to reduce the bacterial contamination of the fresh semen and frozen thawed semen. In the present experiment the preputial capacity was measured 431.33±21.65 ml. On comparing the overall mean, 70% of volume of fluid showed significantly lower bacterial load in comparison to other volume of wash fluid. A significantly ($p<0.05$) higher bacterial load was observed by full volume of fluid.

Keywords: Sahiwal bulls; bacterial load; preputial wash; semen

Introduction

Douching and cleaning of preputial cavity of bulls used for semen collection is very important for quality semen production.

The correct preputial washing at periodic interval is prerequisite for clean semen production but it appears a huge research gap on this issue. Bacterial contamination plays an important role for the future fertility of semen once inseminated in female animals (Griveauet al. 1995; Diemeret al. 1996). Preputial washing reduces the semen microbial contamination to a greater extent without harmful effect on semen quality and preservability. By effective douching and cleaning of prepuce, based on measured capacity of prepuce is of prime importance for quality semen production.Many workers reported preputial douching and cleaning of bulls using 100-200 ml of normal saline solution (NSS) (Bindraet al. 1994) with variable results. It is well established that prepucial capacity may vary from breed to breed and, hence, for optimum douching and cleaning of prepuce of a bull, volume used for douching, may also vary.Higher microbial load in semen is a reflection of unhygienic management in various steps of bull management, semen collection, and processing (Patel et al. 2011).Furthermore, microbial contamination having effects on motility, morphology and various semen quality parameters (Najee et al. 2012; Yanizet al. 2010).

Present experiment is a way forward, the effect of preputial washing on bacterial load of semen and subsequent effect on physico- morphological characteristics of spermatozoa were simultaneously studiedwith aim and objective to determine the comparative efficacy of traditional (Syringe) versus in-house designed PDC device method

Material and Method

The present investigation was carried at the Germ-Plasm Centre, Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly (UP). The institute is located at an altitude of 564 feet above the mean sea level at latitude of 280 North and a longitude of 790East. This place has a subtropical climate with extreme of hot and cold weather condition. The average rainfall of this place is 1087.9 mm and humidity ranging between 15 to 85% in different months of the year.

Calculation of preputial capacity in Sahiwal Bulls

Eleven breeding bull of Sahiwal breed were selected. Preputial hairs were trimmed properly and the area around preputial orifice was cleaned before the start of experiment. Preputial capacity was measured by using Preputial douching and cleaning device (PDC device) developed by this laboratory (Prasad et al., 2016) for the purpose of preputial washing of bulls. After filling of sterilized normal saline solution (NSS) in the water inlet valve (2.5 lits), was closed tightly and then air was pumped into the air inlet valve (30 to 35 psi). The nozzle was then inserted through preputial orifice to a depth of an inch into the preputial cavity. Preputial orifice was kept tightly closed by holding the skin fold tight, to prevent the back flow of the fluid. NSS was then passed to full capacity of preputial cavity while keeping the orifice closed. The nozzle was then withdrawn while keeping the preputial orifice tightly closed. After releasing the grip over the preputial orifice the fluid was drained out and collected into anautoclavable polythene bag. The volume of drained out fluid was measured using graduated measuring cylinder and the actual volume was noted. This technique was repeated three times to each bull of Sahiwal breed.

Experimental animals and nutritional management

A total of 11 healthy breeding bulls of Sahiwal (n = 11; age 2.3 to 4.61 years) were selected for this study. The experimental animals were maintained under identical feeding and managerial conditions during the entire course of the study. The bulls were supplied with concentrate ration (5 kg), green fodder (25 kg) and roughages (5 kg) once a day with ad libitum fresh drinking water

PDC Device

Preputial douching and cleaning device (PDC device) developed by Prasad *et al.* 2016 for the purpose of preputial washing of bulls was used in this present experiment (Fig 1).

Experimental designs

Procedure of Preputial washing- Sterile normal saline solution was prepared as per standard method. A total of three healthy breeding bulls were used to carry out the experiment. Washing of prepuce was done by two methods viz. Traditional or syringe method and PDC device method (Fig.2). In Syringe method (control group), preputial washing was performed with 100 mL NSS using hypodermic syringe (100 mL capacity) fitted with sterilized plastic sheath (6 inches' length) which is a traditional method commonly used at most of the semen collection centres. In PDC device method, three different volumes of NSS was used for the preputial washing i.e. 50%, 70% and 100% of total preputial capacity i.e.431ml (Ankesh Kumar *et al.* 2017). After inserting the desired volume of NSS in the preputial cavity, penile tract of each bull was massaged thrice from downward to upward direction while keeping the orifice closed and then wash fluid was drained out. Since,

same bulls were used in each group hence; an interval of 10 days was given between each experimental method to nullify the effect of preceding method.

Semen sampling

Semen from all the groups were collected on day 1, 5 and 10, and examined, processed and frozen as per standard protocol. Semen samples were also utilized for bacterial load estimation at fresh and post-thaw stage along with their assessment of physico-morphological characteristics (Fig 2).

Fig 1: Preputial douching and cleaning device

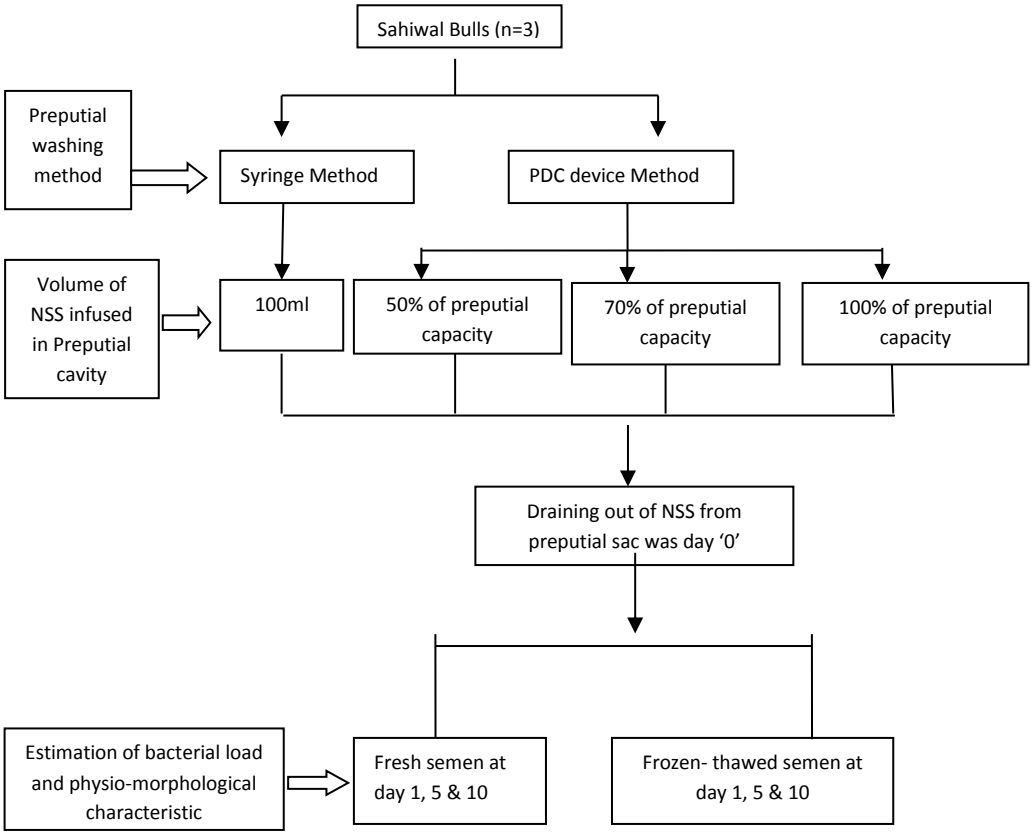

Fig 1: Flow digramme showing experimental design of preputial washing method, volume of NSS infused in preputial cavity, estimation of bacterial load and physico-morphological characteristic of fresh and post-thaw

Standard Plate count (SPC)

The samples processed for standard plate count were collected under strict aseptic condition. Fresh semen samples processed for bacterial counting were used within half an hour of collection while post-thaw semen samples were used after 24h of freezing. Standard plate count was done by spread plate technique in duplicate plates as per Cruickshank et al. 1975. Tenfold serial dilution from 1:10 to 1:1000 were made for all preputial washing samples for standardization of SPC. Subsequently 1:10 to 1:100 dilutions were used for bacterial count from preputial washing samples and 1:10 dilution was used for bacterial count from fresh and post-thaw semen samples. The normal physiological saline solution was used as routine diluents for all the samples. The inoculated nutrient agar plates were allowed to dry for 10 minute before incubation at 37^0C for 24 h. The bacterial count (cfu/ml) of each sample was counted by multiplying the dilution factor with number of colony in plate.

Collection of semen and initial evaluation

Semen was collected from each bull using artificial vagina as per the standard method. Immediately after collection, 200 µl of neat semen was kept separate in the refrigerator (5^0C) for bacterial load estimation and remaining ejaculated semen were placed in water bath at 37^0C. For the purpose of uniformity during entire experiment the ejaculates having mass motility >3+ and having progressive motility > 70% were selected for the study. Fresh semen from all the groups were collected on day 1, 5 and 10 and frozen as per standard protocol.

Mass Motility

The mass activity of the semen sample was determined by placing of fresh semen on clean, grease free slide without cover slip mounted on a stage maintained at 37^0C under low power of microscope. It was graded on the scale of 0 to +5. The semen samples showing mass activity of +3 or above were only utilized for experimental work.

Initial Progressive Motility

The motility was recorded as percentage of progressively motile spermatozoa after the extension of semen. This was assessed by placing a drop of semen diluted with Tris dilutor on a clean, grease free glass slide mounted on a stage maintained at 37^0C and observed under high power objective (20x) after covering with a cover slip. The semen was extended to such an extent that approximately 15-20 spermatozoa were visible under the visual field of microscope. Sample with more than 70% motility only were selected for the study.

Per cent live spermatozoa

The per cent live spermatozoa was determined by adopting differential staining technique using Eosin-Nigrosin stain as described byCampbell et al. 1953

Hypo-Osmotic Swelling Test (HOST)

The HOST was performed as per the method described by Prasad et al. 1999

Results and discussion

Effect of preputial washing on Bacterial load in semen

Fresh stage

The process of increasing the washing volume of fluid increases the elimination of preputial bacterial population thereby decreases the bacterial count in semen of Sahiwal bulls. The observed mean bacterial load of $4.51x10^3$cfu/ml, at day 1 following flushing of 100 ml NSS was comparable to finding by Kher and Dholakia, 1987. Following preputial washing from 100 ml to 430 ml, as the volume of fluid increased, the bacterial count was also changing as the day of the sample increases. The bacterial load was further reduced from 3.01 x 10^3 to 2.00 x 10^3cfu/ml, at day 1 following increasing of washing volume from 215 ml to 300 ml, was comparable to report ($4.9x10^3$ cfu/ml in Gir Bulls) by Kher and Dholakia, 1987. Similar trend in bacterial count were also observed at day 5 and day 10. It is possible that the excess volume of fluid like the total preputial capacity caused wound because of pressure injury to tissue.This may lead to the invasion of exogenous bacteria, because exposure of subcutaneous tissue following loss of skin integrity (*i.e.*, a wound) provides a moist, warm and nutritious environment conducive to microbial colonization and proliferation (Bowler et al. 2001) resulting in to the increased bacterial count (Table 1).

Post- thaw

At Post-thaw stage at day 1 also showed a similar trend as observed in fresh semen following washing. It also revealed a decreasing ($p<0.05$) trend in the bacterial count with the increase of washing fluid volume except 430 ml volume in which count was increased significantly ($p<0.05$) in the same fashion as in fresh semen. Similar trends were also recorded at day 5 with little changes at day 10. Following flushing of 100 ml NSS, the observed mean bacterial load of $2.96x10^3$cfu/ml, at day 1, was not in conformity to finding by Bindraet al, 1994; Meenaet al. 2015 by using 100 ml NSS. Further, on increasing of washing fluid from 100 ml to 215 ml, the count was reduced from $2.96x10^3$ to 2.10 x 10^3cfu/ml, which was also not in conformity with finding by Ramaswamyet al. 1994 who reported $1.3x10^3$cfu/ml without washing. Similar trends were also recorded at day 5

and 10.Based on present study, this could be concluded that using 70% fluid volume of preputial capacity may be a most suitable volume of preputial washing helpful in reducing bacterial load in fresh and post thaw semen of Sahiwal bulls (Table 2).

Effect of preputial washing on physico- morphological characteristics of spermatozoa in Sahiwal bulls

Mass Motility

Following preputial washing, at day 1, 5 and 10, there has not been any significant differences were recorded either among the day or among the methods, while on comparing overall mean, 70% volume of wash fluid showed significantly ($p<0.05$) increased in mass motility in comparison to other volume of fluid. Study also showed a volume effect on mass motility with a linear relationship with the increasing of the volume of washing fluid from increases the mass motility.

Individual Progressive motility

Fresh Stage

In syringe method, following preputial washing, significant changes observed in progressive motility of fresh semen among the days. The observed per cent progressive motility of 75.38±0.47, at day 1 in this experiment is not conformity with finding using 100 ml NSS by Bindraet al. 1994 (83.83±2.11). In PDC device method, there has been observed the significant differences in progressive motility among the days. In the present experiment, in general, the higher progressive motility percent mostly observed at day 1 in comparisons to other day following preputial washing. The obtained individual progressive motility per cent at day 1 following washing of this study was not in conformity to report of Bindra*et al.*1994 who obtained 88.83±2.11 using 100 ml NSS in Murrah bulls. Further, when we increased the volume of washing fluid from 215 ml to 300 ml, the motility per cent remained same. But when, when the volume of fluid was further increased up to the total capacity *i.e.* 430 ml, the motility per cent was significantly decreased ($p<0.05$), which was a reverse in the trend. It is possible that the volume of fluid corresponding to the total preputial capacity caused wound because of pressure injury to tissue (Table 3).

Post-thaw stage

Following preputial washing, in syringe method there has been observed significant ($p<0.05$) changes in progressive motility among the days. The higher motility of 45.85±0.59 was observed at day 1 and 10. The obtained motility in this experiment is not consistent with finding of Meena et al. 2015. In PDC device method, when the volume of washing fluid increased from 100 ml to 215 ml, the progressive motility per cent characteristically increased from 45.85±0.59 to 52.49±1.10, which was comparable to previously report by Meena et al. 2015 (52.50±2.14) using 100 ml sterile NSS in Murrah bulls. On the other hand, when the volume of fluid was further increased up to the total capacity *i.e.* 430 ml, the progressive motility per cent was significantly decreased, which was a reverse in the trend. It is possible that the volume of fluid corresponding to the total preputial capacity caused wound because of pressure injury to tissue (Table 4)

Per cent live spermatozoa

Fresh Stage

In syringe method, there was significant ($p<0.05$) differences in liveability of spermatozoa among the days. The higher per cent viability of sperm was observed on day 1 (81.27±0.03) and 5 (79.49±0.59). The obtained viability in the present experiment is not conformity with the finding of Bindra et al. 1994 (88.66±1.26). In PDC device, at day 1 the significant difference in per cent liveability was observed as the volume of flush fluid increases. The observed motility with the wash volume of 215 ml was not in conformity with finding of Bindra et al. 1994. When the volume of fluid further increased from 215 ml to 300 ml, non-significant difference was observed. On the other hand, when the volume of fluid was further increased up to the total capacity *i.e.* 430 ml, the per cent viability was significantly decreased. The possible reasons of these changes have already been mentioned in the previous section. Nearly similar trend of liveability was also observed on day 5 and 10.

Post-thaw stage

In syringe method, a non-significant difference was observed among the days. However, the observed liveability per cent of 55.17±0.51 at day 1 was not consistent with the finding of Meena et al, 2015; Bindra et al. 1994 using 100 ml of NSS. In PDC device method at day 1, the significant difference in per cent liveability was observed among the method. The present finding is not in conformity with the finding by Bindra et al. 1994. However, a positive correlation was observed between volume of flushing and liveability of spermatozoa, as the volume of fluid increased liveability also increased with exception to full capacity in which the per cent liveability decreased significantly ($p<0.05$)(Table 5).

Table 1: Effect of preputial washing on bacterial load ($x10^3$cfu/ml) in fresh semen samples using Syringe and PDC device method in Sahiwal bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day 1	Day 5	Day 10	
Syringe Method	100 ml (Control)		4.51 ± 0.04^{A}	4.65 ± 0.07^{A}	4.91 ± 0.04^{B}	4.69 ± 0.06^{A}
PDC device method	215 ml	50%	3.01 ± 0.13^{Bb}	3.67 ± 0.16^{Ba}	4.16 ± 0.27^{Ca}	3.61 ± 0.19^{B}
	300 ml	70%	2.00 ± 0.01^{Cb}	2.90 ± 0.15^{Ca}	3.43 ± 0.08^{Da}	2.77 ± 0.21^{C}
	430 ml	100%	4.61 ± 0.27^{Ab}	5.00 ± 005^{Aab}	5.56 ± 0.03^{Aa}	5.06 ± 0.13^{A}

Means bearing different superscripts (a, ab and b) differ significantly in a row ($p<0.05$); Means bearing different superscripts (A, B, C and D) differ significantly in a column ($p<0.05$)

Table 2: Effect of preputial washing on bacterial load ($x10^3$cfu/ml) in post-thaw semen samples using Syringe and PDC device method in Sahiwal Bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day 1	Day 5	Day 10	
Syringe method	100 ml(Control)		2.96 ± 0.18^{Ab}	3.46 ± 0.23^{Aab}	3.90 ± 0.05^{Aa}	3.44 ± 0.16^{A}
PDC device method	215 ml	50%	2.10 ± 0.15^{Bb}	2.53 ± 0.16^{Bab}	3.20 ± 0.05^{Aa}	1.17 ± 0.13^{B}
	300 ml	70%	0.73 ± 0.08^{Cb}	1.20 ± 0.15^{Cab}	1.60 ± 0.05^{Ba}	1.77 ± 0.13^{C}
	430 ml	100%	3.20 ± 0.15^{Ab}	3.80 ± 0.17^{Aab}	4.46 ± 0.06^{Aa}	3.82 ± 0.19^{A}

Means bearing different superscripts (a and b) differ significantly in a row ($p<0.05$); Means bearing different superscripts (A, B and C) differ significantly in a column ($p<0.05$)

Table 3: Effect of preputial washing on per cent individual progressive motility of fresh semen ejaculate using Syringe and PDC device method in Sahiwal Bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day 1	Day 5	Day 10	
Syringe Method	100 ml (Control)		75.38 ± 0.47^{Ba}	74.33 ± 0.06^{Bab}	73.20 ± 0.04^{Cb}	74.30 ± 0.34^{C}
PDC device method	215 ml	50%	81.41 ± 0.16^{Aa}	79.04 ± 0.54^{Ab}	76.14 ± 0.24^{Bc}	78.86 ± 0.78^{B}
	300 ml	70%	81.94 ± 0.40^{Aa}	80.52 ± 0.14^{Aab}	79.65 ± 0.23^{Ab}	80.70 ± 0.36^{A}
	430 ml	100%	75.09 ± 0.38^{Ba}	73.42 ± 0.05^{Bb}	72.81 ± 0.18^{Cb}	73.77 ± 0.36^{C}

Means bearing different superscripts (a, b and c) differ significantly in a row ($p<0.05$); Means bearing different superscript (A, B and C) differ significantly in a column ($p<0.05$)

Table 4: Effect of preputial washing on per cent individual progressive motility of post thaw semen ejaculate using Syringe and PDC device method in Sahiwal bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Days 1	Day 5	Day 10	
Syringe Method	100 ml (Control)		45.85 ± 0.59^{Ca}	42.20 ± 1.00^{Ba}	41.15 ± 0.80^{Ab}	43.07 ± 0.82^{BC}
PDC device method	215 ml	50%	52.49 ± 1.10^{Ba}	46.15 ± 1.43^{Ab}	40.77 ± 0.50^{Ac}	46.47 ± 1.77^{AB}
	300 ml	70%	60.27 ± 1.05^{Aa}	48.80 ± 1.59^{Ab}	40.76 ± 0.93^{Ac}	49.94 ± 2.89^{A}
	430 ml	100%	43.47 ± 0.06^{C}	42.17 ± 0.46^{B}	41.26 ± 0.40^{A}	42.30 ± 0.36^{C}

Means bearing different superscripts (a, b and c) differ significantly in a row ($p<0.05$); Means bearing different superscripts (A, B and C) differ significantly in a column ($p<0.05$)

Table 5: Effect of preputial washing on per cent liveability of post-thaw semen ejaculate using Syringe and PDC device method in Sahiwal bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day1	Day 5	Day 10	
Syringe Method	100 ml (Control)		55.17 ± 0.51^{C}	54.17 ± 0.51^{B}	53.28 ± 0.76^{B}	54.21 ± 0.40^{B}
PDC device method	215 ml	50%	58.29 ± 1.14^{Ba}	55.84 ± 1.52^{Ba}	51.87 ± 1.16^{Bb}	55.33 ± 1.13^{AB}
	300 ml	70%	61.43 ± 0.13^{Aa}	58.74 ± 0.72^{Ab}	56.34 ± 0.06^{Ab}	58.83 ± 0.76^{A}
	430 ml	100%	48.03 ± 1.68^{Da}	45.24 ± 0.8^{Cab}	43.27 ± 0.57^{Cb}	45.51 ± 0.89^{C}

Means bearing different superscripts (a and b) differ significantly in a row ($p<0.05$); Means bearing different superscripts (A, B and C) differ significantly in a column ($p<0.05$).

Table 6: Effect of preputial washing on per cent HOS response of fresh semen ejaculate using Syringe and PDC device method in Sahiwal Bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day 1	Day 5	Day 10	
Syringe Method	100 ml (Control)		63.93 ± 0.42^{BCa}	62.05 ± 0.34^{BCab}	60.54 ± 0.25^{Bb}	62.17 ± 0.52^{C}
PDC device method	215 ml	50%	65.69 ± 0.81^{Ba}	64.60 ± 0.42^{Bab}	62.46 ± 0.14^{ABb}	64.25 ± 0.54^{B}
	300 ml	70%	75.95 ± 0.83^{Aa}	69.93 ± 1.45^{Ab}	65.37 ± 0.32^{Ac}	70.41 ± 1.60^{A}
	430 ml	100%	62.36 ± 0.16^{Ca}	60.79 ± 0.39^{Cab}	58.77 ± 0.89^{Bb}	60.64 ± 0.59^{C}

Means bearing different superscripts (a and b) differ significantly in a row ($p<0.05$); Means bearing different superscripts (A, B and C) differ significantly in a column ($p<0.05$)

Table 7: Effect of preputial washing on per cent HOS response of post-thaw semen ejaculate using Syringe and PDC device method in Sahiwal bulls

Method of preputial washing	Volume of fluid		Days			Overall Mean±SE
			Day 1	Day 5	Day 10	
Syringe Method	100 ml (Control)		48.07 ± 0.46^{Ba}	45.81 ± 0.17^{Bab}	44.49 ± 0.49^{Bb}	46.12 ± 0.56^{B}
PDC device method	215 ml	50%	52.56 ± 0.59^{Aa}	50.54 ± 0.10^{Aab}	47.72 ± 0.27^{Ab}	50.275 ± 0.72^{A}
	300 ml	70%	54.90 ± 0.65^{Aa}	52.87 ± 0.97^{Aab}	50.35 ± 1.20^{Ab}	52.70 ± 0.81^{A}
	430 ml	100%	43.97 ± 0.89^{Ca}	42.01 ± 0.42^{Cab}	40.64 ± 0.47^{Cb}	42.20 ± 0.57^{C}

Means bearing different superscripts (a and b) differsignificantly in a row ($p<0.05$); Means bearing different superscripts (A B and C) differ significantly in a column ($p<0.05$)

Hypo-osmotic Swelling Test (HOST)

Fresh stage

In syringe method, a significant ($p<0.05$) difference was observed among the days. The highest HOS responsive sperm was observed on day 1 (63.93±0.42) and day 5 (62.05±0.34). In PDC device, at day 1, a significant difference was observed among the method, when the volume of fluid increased from 100 ml to 215 ml, no change was observed. When volume of fluid increases from 215 ml to 300 ml the HOST per cent increased from 65.69±0.81 to 75.95±0.83 per cent. Furthermore, increasing of washing volume from 300 ml to 430 ml, reverse in trend of HOS response was observed. The reason of the non-significant difference between 100 ml and 215 might be inadequate quantity of flushing volume to clean the prepuce efficiently, while a significant difference in HOST percentage observed between 215 ml and 300 ml of wash volume may be a proper volume to clean the prepuce in comparison to other flushing volume, while it is exception to 100% (Full volume) where the HOST per cent was observed in decreasing trend. The reason of this reverse trend may be same as it already mentioned in previous section. Nearly similar response was also observed at day 5 and 10. (Table No. 6)

Post-thaw stage

In syringe method, a significant difference was observed in HOS responses among the days. The higher HOS responses in this present experiment is comparable with report by Meena et al. 2015 who observed 40.33±3.88 per cent of HOS reactive sperm using 100 ml NSS. In PDC device method, a significant difference was observed among the method. When the volume of fluid increased from 100 ml to 215 ml a significant increase in HOS responses were observed, while on further increasing of volume a non-significant changes in HOS positive sperm was observed. On the other hand, a reverse trend of HOS response was observed as usual when the volume fluid increase to the full capacity of prepuce. Nearly similar trend of this was also observed at day 5 and 10. No sufficient literature could be traced to compare our findings. (Table No.7)

Summary and Conclusions

In fresh and post-thaw semen, the process of increasing the washing volume of fluid increases the elimination of preputial bacterial population thereby decreases the bacterial count in semen. Following preputial washing from 100ml to 430ml, as the volume of fluid increased, the count was also changing as the day of the samples increases. The lower bacterial load in this present study usually recorded on day 1 in comparison to other day regardless of flushing volume. The bacterial load was reduced. A significantly ($P<0.05$) lower bacterial load was observed with 70% method in comparison to other method and that remained significantly lower at day 5 and 10 in fresh as well as in post-thaw semen. At the level of physico-morphological examination of spermatozoa of semen at fresh and frozen stage, effect of flushing was significantly correlated to the increases volume of fluid except at full volume of fluid. Following flushing with 70% and 50% method of PDC device, the observed progressive motility, liveability and HOST were significantly ($P<0.05$) higher with significantly lower ($P<0.05$) in sperm abnormality of semen sample of the day 1 followed by day 5 and 10.

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