Himachal Journal of Agricultural Research 46 (1): 91-94(2020)

Prevalence of bacteria in different stages of semen processing before and after semen collection of cow bulls of Himachal Pradesh

Pankaj Rana* and Madhumeet Singh[1]
Department of Animal Husbandry
Veterinary Hospital, Chururu, District Una-177 211, India.
[1]Department of Veterinary Gynaecology and Obstetrics, CSK HPKV, Palampur.

*Corresponding author: 94ranapankaj@gmail.com
Manuscript received: 13.06.2020; Accepted: 30.06.2020

Abstract

The present study was conducted to find prevalence of bacteria in various stages of semen processing before and after semen collection and among egg yolk and semen extender added to the semen. A total of 128 samples (30 each of preputial washings, neat, extended and frozen cow bull semen) were collected out of which 97 samples showed bacterial growth. The prevalence of bacteria in preputial washings, neat semen, extended semen, semen straw, extender, egg yolk was found to be 100, 100, 73.33, 43.33, 0, 25 per cent, respectively.

Key words: Preputial washings, neat semen , extended semen, frozen semen.

India is highest milk producer in the world since 1997 having 176.4 million tonnes of milk production in the year of 2017-18. Per capita availability of milk has increased to 374 grams per day in 2017-18 which was 130 grams per day in 1950-51 (DAHD 2018). Although India holds first position in terms of milk production but productivity per animal is low and can be attributed to unutilized potential of superior and hygienic male germplasm. The importance of improved germplasm of male animal is still untapped (Meena 2008).

Presently, around 85 million frozen semen doses (FSDs) are being produced per year in 44 A and B graded semen stations of the country to fulfill the requirement for Artificial insemination (AI) in the country. The target of frozen semen production for 2021-2022 has been set to 140 million FSDs. In the year of 2014-2015, 482.63 lakhs frozen doses were produced by government semen station across the country, 151.67 lakhs by dairy co-operatives, 233.10 lakhsby NDDB and 103.78 lakhs by private semen station (Rath et al. 2016). With more organized dairy farming, the demand for Artificial Insemination (AI) and thereby for frozen semen is expected to rise in the near future.

Semen produced from infected bull and unhygienic processing will only lead to artificial introduction of infection. Bacteria can decrease the fertility of the bull by causing disease of the reproductive tract or by affecting spermatozoa to prevent fertilization (Givens and Marley 2008). The procedure of semen collection invites bacterial contamination through various sources (Meena 2008). Bacteria gain access into semen from diseased animals, preputial cavity, dilutors and unhygienic practices followed during collection, processing and packaging of semen. Regular evaluation of semen samples is a prerequisite to ensure the quality of semen. Bio-security and disease transmission across countries are also important concerns over the use of egg yolk based extenders since they facilitate the transmission of diseases (Singh *et al.* 2017)

Materials and Methods

The present study was carried out from July 2018 to January 2019 at Sperm Station, Himachal Pradesh Livestock Development Board, Palampur (1472 meters above the mean sea level, longitude 32.1109°N, latitude 76.5363°E) and at Department of Veterinary Gynaecology and Obstetrics, College of Veterinary & Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The study was conducted on fifteen adult healthy bulls (13 Jersey and 2 Red Sindhi) aged between two to six years maintained at Sperm Station, Himachal Pradesh Livestock

Development Board, Palampur. Samples were collected twice from each bull at weekly interval. Tris egg yolk buffer was used for extending the semen at room temperature (20-22°C) and it was frozen by programmed freezing method.

Overall, 128 samples were collected comprising of preputial washings (n=30), neat semen at collection (n=30), extended semen before freezing at dilution at 34^0C (n=30), frozen semen straws (n=30), Extender (n=4) and egg yolk (n=4). Following the collection of various samples under aseptic conditions, these were transported to microbiological laboratory of Department of Veterinary Gynaecology and Obstetrics, Palampur in Freezer box as soon as possible. These samples were subjected for further procedures in laboratory.

About 20 ml of NSS was introduced into the preputial sheath by a sterilized AI sheath and a sterile 20 ml syringe. Further by closing the orifice, the preputial sheath was massaged and preputial washings were aspirated. After discarding the first few drops, rest was transferred into a sterile sample collection tube. The neat semen sample were obtained insterile screw capped vial (0.2 to 0.5 ml) collected through artificial vagina adopting the routine procedure of collection through veterinary officer at the semen collection station. Extended semen samples, each 1 ml, were obtained separately at the semen collection station. The frozen semen straws of different batches containing 0.25 ml of frozen semen per straw were procured and transported to laboratory in cryocan.

Results and Discussion

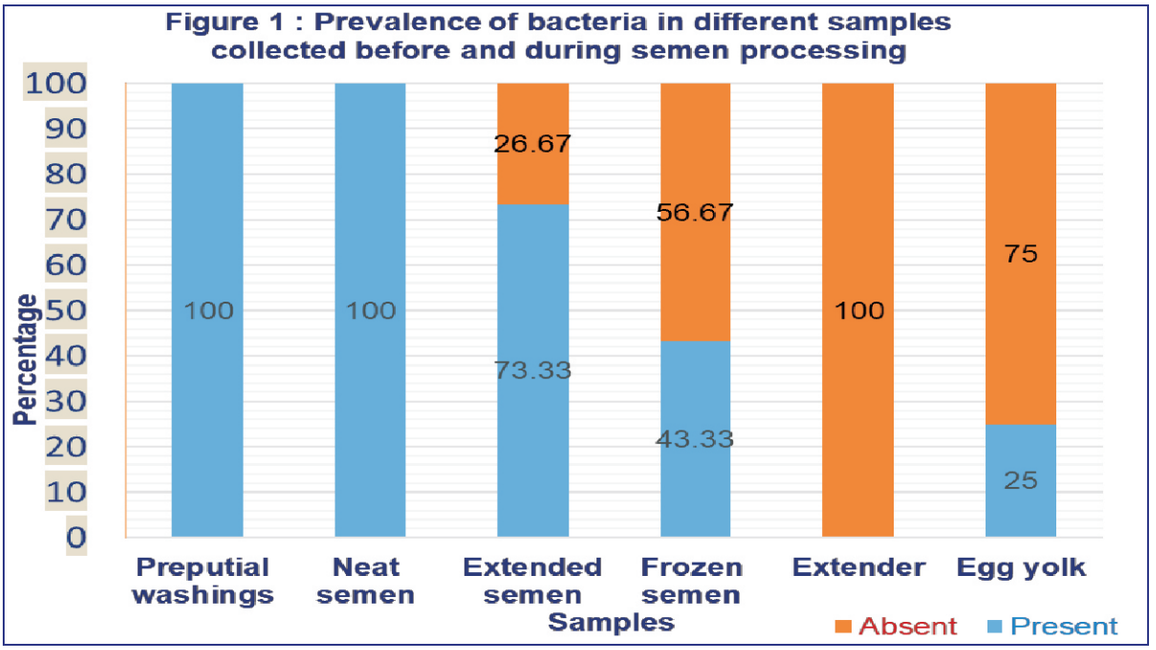
Prevalence of bacteria in different samples collected from Sperm station, Palampur has been shown in Table 1 and Figure 1.

Appraisal of Table 1 shows that the prevalence of bacteria ranged from 0 (Extender) to 100 per cent (preputial washings and neat semen). High prevalence of bacteria in preputial washings and neat semen in our study is in accord with the fact that preputial cavity harbors a large number of saprophytic microflora (Paray *et al.* 2018). As per studies conducted by Aurich and Spergser (2007), whereas, commonly prepuce harbors nonpathogenic bacteria, it may also provide niche for pathogenic organisms. Preputial orifice forms the opening of preputial cavity. Since, preputial cavity is capable of considerable dilation; therefore, it can be easily contaminated by surrounding environment.

High prevalence of bacteria in neat semen can be attributed to the various sources of bacterial contamination in neat semen viz. preputial washings (Meena *et al.* 2015) and infection of reproductive tract (Bielansk 2007). Some sources of bacterial contamination in neat semen can be AV assembly, lubricants, test tube, gloves of handler, dummy animals and semen collection tube cork Sannat *et al*. (2015). Earlier, Althouse and Lu (2005) had classified sources of bacterial contamination in extended ejaculates of boar semen into animal and non-animal origin. Faecal contaminants, preputial fluids, skin and preputial hairs were identified as sources of animal origin while sources of non-animal origin included non-sterile water, feed, beddings, ventilation system, sinks, drains and inanimate objects. Similarly, Singh (2018) concluded that any stage of semen collection and processing is prone for bacterial contamination but the stage of semen collection is the most susceptible for bacterial contamination. Flow of preputial fluid, long preputial hairs and dirty collection glove in boar have been reported to increase bacterial contamination of semen (Goldberg 2013).

Table 1. Prevalence of bacteria in different samples collected before and during semen processing

Sr. No.	Sample	Number (n)	Samples positive for bacteria		Samples devoid of bacteria		Total No. of bacterial isolates
			n	%	n	%	
1.	Preputial washings	30	30	100	0	0	71
2.	Neat semen	30	30	100	0	0	62
3.	Extended semen	30	22	73.33	8	26.67	27
4.	Semen straw	30	13	43.33	17	56.67	22
5.	Extender	4	0	0	4	100	Nil
6.	Egg yolk	4	1	25	3	75	1



In our study, the prevalence of bacteria in extended semen was found to be 73.33 per cent. Studies conducted by Althouse (2008) concluded that the composition of extender can provide nutrition to the bacteria. Schulzea *et al.* (2014) isolated bacteria from neat and extended semen and found that bacteria isolated from extended semen were different than bacteria isolated from neat semen signifying contamination while processing of semen. In a study conducted by Meena *et al.* (2010) it was concluded that bacterial load in traditional egg based extender is more than biociphos extenders.

In present study, the prevalence of bacteria in frozen semen was found to be 43.33 per cent. Thibier and Guerin (2000) reported that liquid nitrogen may also spread bacterial agents. Frozen semen can be infected by equipment of cryopreservation chain (Rao *et al.* 2014). However, infected semen straws and tank does not spread the infection to other non-infected properly sealed semen straws (Bielansk 2007). Grout and Morris (2009) concluded that although at production liquid nitrogen has negligible bacterial load but it can invite bacterial contamination during handling and storage. In their study, Morris (2005) concluded that ice crystals originate from open air and therefore, can serve as medium for bacterial contamination in frozen semen. Another study conducted by Meena (2008) concluded that no significant difference was recorded in the bacterial count of the cryopreserved semen stored for 5, 10, 15 and 20 years. Prevalence of bacteria in egg yolk was found to be 25 per cent while extender samples were sterile. Low prevalence rates of both of these samples can be attributed to hygienic practices of the sperm station.

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