

Semen Discarded During Different Stages of Cryopreservation in Ongole (*Bos indicus*) Bulls

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Abstract

Indian subcontinent is a treasure house of *Bos indicus* cattle, one of the most popular breed amongst them is "Ongole" which by inheritance is a dual purpose breed. During the period under report 549 ejaculates were collected from eight breeding bulls, of which 192 ejaculates (34.97%) were discarded during various stages of semen processing. Out of the total ejaculates, 126 (22.95%), 40 (7.29%) and 26 ejaculates (4.74%) were discarded after collection, equilibration and freezing, respectively while most of the discards were second ejaculates. From the present study, it was concluded that the ejaculate discard in Ongole bulls was mainly based on low volume, initial progressive motility, sperm concentration and post-thaw motility. However, it was suggested that certain reproductive technologies like Sephadex or Glass wool filtration or Percoll gradients could be used to enhance the quality of these low grade ejaculates from genetically superior bulls.

Keywords: Cryopreservation; *Bos indicus*; Ongole bulls; Ejaculate discard

Introduction

Indian subcontinent is a treasure house of *Bos indicus* cattle, one of the most popular breed amongst them is "Ongole" which by inheritance is a dual purpose breed well known for its thriftiness, hardiness and disease resistance. It has been observed that in indigenous as well as crossbred bulls a huge percentage of ejaculates were discarded due to low motility, high proportion of abnormal sperms and poor freezability during various stages of semen freezing [1,2]. There might be species differences in overall sperm sensitivity to cryopreservation; the ejaculate was heterogeneous with a variable resistance to osmotic stress amongst the cells [3]. Under tropical conditions, exotic breeds showed significantly seasonal fluctuations in semen characteristics as high ambient temperature during summer adversely affected the testicular size, libido and semen quality and epididymal spermatozoa by elevated testicular temperature with decreased the ability of spermatozoa to maintain motility and acrosomal integrity after freezing [4]. In comparison to fresh semen, 8 times more cryopreserved bovine sperms were required to achieve equivalent fertilization rates in vivo [5]. Mechanization of agricultural practices and introduction of cross breeding programme lead to a drastic decline in the population of the pure Ongole bulls. Ultimately, selection of breeding bulls was dependant on phenotypic characters as availability of good pedigreed bulls was limited. Hence, the current situation demands studies on the factors responsible for semen discards and/or methods to utilize the discarded ejaculates by adopting suitable assisted reproductive technologies to exploit the available germplasm to the fullest potential. For effective utilization of superior germplasm it was necessary to minimize the ejaculate discard rate [6].

For breeding bulls used in artificial insemination, the information on semen characteristics, fertility and semen production efficiency are some of the important basic parameters, such information in Ongole bulls is lacking. Perusal of literature revealed paucity of information on indigenous breeding bulls especially Ongole breed pertaining to the number of ejaculate discard rate during various stages of semen freezing.

Material and Methods

Semen was collected from 8 breeding bulls, once in a week with the standard artificial vagina using an anoestrus cow as a dummy during the period under report from January 2009 to March 2010. Each time two ejaculates were collected at a gap of 20 to 30 minutes after allowing one or two false mounts. During the period under report 549 ejaculate were collected. Soon after the collection, the semen tubes were numbered and kept in a water bath at 37°C and transferred to the laboratory for further evaluation and processing. The criteria adopted to accept fresh semen for further processing was that it should possess mass activity of atleast ++ and above, initial progressive motility of more than 70% and sperm concentration of not less than 500 million per ml of neat semen. The samples were diluted with TRIS fructose citric acid egg yolk glycerol (8%) dilutor based on the number of viable sperm and concentration such that a minimum concentration of 30 million sperms per dose of 0.5 ml was available. The diluted semen was treated at 5°C for 6 h as equilibration period. A sample of diluted semen from each bull was accepted if the pre freeze progressive motility was a minimum of 60%. The diluted semen was filled in medium sized (0.5 ml) French straws and frozen in liquid nitrogen vapours (rapid horizontal freezing) adopting standard freezing protocol. The frozen semen straws were thawed at 37°C for 30 sec after 24 h post freezing and evaluated of post thaw progressive motility

minimum acceptable progressive motility of 40% [7]. Semen doses which had below 40% post thaw progressive motility were discarded. The number of ejaculates discarded at various stages of cryopreservation were tabulated and discussed.

Results and Discussion

Ejaculates discarded at various stages of cryopreservation in Ongole bulls were tabulated in Table 1. During the period under report 549 ejaculate were collected from eight breeding bulls of which a total of 192 ejaculates (34.97%) were discarded during various stages of semen processing. Out of the total ejaculates, 126 (22.95%), 40 (7.29%) and 26 ejaculates (4.74%) were discarded after collection, equilibration and freezing, respectively while most of the discarded were second ejaculates. These findings are in agreement with the observations of Wright [8], Mishra [2], Tiwari et al. [4] and Shrivatava et al. [6] who also reported that the discard rate ranged between 8 to 10%. While, Anchieta et al. [9] reported that undesirable physical characteristics of semen in Zebu and morphological characteristics in European breeds were the major cause for discarding ejaculates. They further observed that, the percent of ejaculates discarded at various stages of cryopreservation varied between 23.88 to 60.56 among the different breeding bulls. Although the bulls were considered as a homogeneous group, the differences in number of semen ejaculates among bulls may be attributed to the variations in the sexual arousal, secretory activities of the accessory sex glands, scrotal circumference, and age and body weight [10]. More number of ejaculates were discarded after collection as majority of these ejaculates had either abnormal colour and odour, low mass activity, initial progressive motility and sperm concentration, while some ejaculates also contained debris/foreign material which

might be attributed to the presence of a pendulous preputial sheath in Ongole bulls. Although, studies on seasonal variation in the ejaculate discard rate was beyond the scope of the present study, it was observed that poor quality ejaculates were recorded more during winter months compared to other seasons. These findings could be attributed to the breed difference as Ongole breed belonged to the tropical climatic zone and the other being presence of less/no scrotal fat that was needed for insulation to the testes during winter [11]. On the contrary, Tiwari et al. [4] observed more discards during summer followed by rainy seasons in Red Sindhi breed, which might be due to the variation in the location of native tract of that breed. Relatively low numbers of ejaculates were discarded after equilibration period (7.29%) and during freezing (4.74%) which were mainly due to cold shock, poor freezability, osmotic stress, and post thaw progressive motility. Similar, observations were recorded by Tiwari et al. [4] in Red Sindhi bulls while Shrivatava et al. [6] found a similar trend in HF crossbred bulls. Ejaculates discarded after equilibration and freezing might be due to damaging stresses of the cryopreservation protocol: firstly, the change in temperature; secondly, the osmotic and toxic stresses presented by exposure to molar concentrations of cryoprotectants; and thirdly, the formation and dissolution of ice crystals in the extracellular environment as opined by Watson [3]. Further, some workers have opined that at high dilution levels, as for bull semen, sperm function declines due to a phenomenon called as “dilution effect”, which may be due to reduced contact between sperm and components of the male reproductive tract [12]. While cryoprotectant agents are essential for cell survival during cryopreservation, their addition and subsequent removal creates an anisoosmotic environment for the cells, resulting in osmotically driven volume excursions and potential osmotic damage [13].

Bull No	Ejaculates Collected	Ejaculates Discarded							
		Collection		During Equilibration		After Freezing		Total Ejaculates Discarded	
		No	%	No	%	No	%	No	%
408	61	14	22.95	3	4.92	2	3.28	19	31.15
409	65	13	20.00	3	4.62	2	3.08	18	27.69
417	73	18	24.66	6	8.22	0	0.00	24	32.88
424	71	15	21.13	1	1.41	3	4.23	19	26.76
426	67	11	16.42	2	2.99	3	4.48	16	23.88
465	71	20	28.17	12	16.90	11	15.49	43	60.56
1011	66	19	28.79	8	12.12	3	4.55	30	45.45
1027	75	16	21.33	5	6.67	2	2.67	23	30.67
Total	549	126	22.95	40	7.29	26	4.74	192	34.97

Table 1: Ejaculates discarded at various stages of semen processing in Ongole bulls.

It was summarized from the present findings that the proportion of spermatozoa, which could survive the cryopreservation protocol, was determined by the sensitivity to osmotic stress during cryopreservative addition, during cooling and freezing leading to an abrupt change from the gel to the liquid-crystal phase of membrane phospholipids, causing irreversible structural changes to the membrane architecture and increased permeability. As the sperms are

frozen, ice crystals are formed in the extracellular medium, increasing the osmolality of the unfrozen water, intracellular water diffuses out, thus dehydrating the cell and plasma membrane that is lethal to the sperm cell. It seems that frequency of ejaculate discard recorded in present study was inevitable. Further, it was concluded that the ejaculate discard in Ongole bulls was mainly based on low volume, initial progressive motility, sperm concentration and post thaw

motility. However, it was suggested that certain reproductive technologies like Sephadex or Glass wool filtration or Percoll gradients could be used to enhance the quality of these low grade ejaculates from genetically superior bulls [14]. Further, it was opined that the sperm attributes and rate of discard in relation to season are to be studied in detail for effective utilization of superior germplasm.

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