EFFECT OF NON-GENETIC FACTORS ON THE QUALITY OF FROZEN SEMEN OF CROSSBRED HOLSTEIN FRIESIAN BULLS

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ABSTRACT

Sixty five frozen semen samples of 14 crossbred Holstein Friesian bulls were subjected to quality assurance tests such as post-thaw motility, post-thaw incubation survival, hypo-osmotic swelling (HOST) and acrosome integrity. The data were statistically analyzed to study the effect of farm (I and II), ejaculate (I and II), period (2007-2014) and season (summer, winter and northeast monsoon) on the frozen semen quality using general linear model. Post-thaw motility and post-thaw incubation survival of spermatozoa was highly affected ($p<0.01$) by ejaculate, period and season, whereas, HOST was affected by period, and acrosome integrity was affected by ejaculate and period. In brief, non-genetic factors had impact on the quality of frozen semen of crossbred Holstein Friesian bulls.

Keywords: Acrosome integrity, Frozen semen, Hypo-osmotic swelling, Non-genetic, Post-thaw motility

INTRODUCTION

The evaluation of frozen semen is a mandatory procedure in all the semen production stations to assess the quality of frozen semen produced before distribution of semen straws for artificial insemination. During freezing of semen, the changes in structure and functional abilities of spermatozoa would lead to poor fertilization (Kasimanickam *et al*., 2006). Each ejaculate of a bull has the heterogenous population of spermatozoa and hence the functional alterations vary from impaired motility to death of spermatozoa. Therefore, the post-thaw sperm motility, plasma membrane integrity and status of acrosomes are the most important and pertinent parameters to assess the quality of frozen thawed spermatozoa. These parameters are influenced by genetic and non-genetic factors. In this paper, the effect of non-genetic factors on the quality of frozen semen of crossbred Holstein Friesian bulls was studied.

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MATERIALS AND METHODS

Sixty five frozen semen samples were collected from 14 crossbred Holstein Friesian bulls stationed in two frozen semen stations during the period from 2007 to 2014. The frozen semen samples were subjected to quality assurance tests to evaluate post-thaw motility, post-thaw incubation survival, hypo-osmotic swelling and acrosome integrity of spermatozoa. The collected data were analyzed using SPSS 17.0 software to study the effect of non-genetic factors viz. farm (I and II), ejaculate (I and II), period (I - 2007 to 2008, II - 2009 to 2010, III - 2011 to 2012 and IV - 2013 to 2014) and season (Winter - December to February; Summer - March to May; Northeast monsoon - September to November) by univariate statistical analyses under general linear model, $Y_{ijkln} = \mu + F_i + E_j + S_k + P_l + e_{ijkln}$, where, Y_{ijkln} is an observation on the n^{th} bull belonging to i^{th} farm, j^{th} ejaculate, k^{th} season and l^{th} period; μ is the overall mean; F_i is the fixed effect of i^{th} farm (i =1 to 2); E_j is the fixed effect of j^{th} ejaculate (j = 1 to 2); S_k is the fixed effect of k^{th} season of semen collection (k = 1 to 4) and P_l is the fixed effect of l^{th} period (l = 1 to 4) of semen collection and e_{ijkln} is the residual random

error, NID (0 and σ^2_e). To study the influence of farm, ejaculate, season and period on post-thaw motility and post-thaw incubation survival at different time intervals (0h, 1h, 2h and 3h), the statistical technique of repeated analysis of variance model was used.

For assessing post-thaw motility and post-thaw incubation survival of spermatozoa, the frozen semen straw was thawed in a water bath at 37ºC for 30 sec. Thereafter, the straw was wiped with cotton to remove water particles adhered on the outer surface of straws and thawed semen was collected in a test tube by cutting ends of the straw. By using a pipette, a drop of thawed semen was placed on clean, grease-free glass slide and was covered with a cover slip without any air bubbles, followed by examination under phase contrast microscope (40X) to estimate the post-thaw motility (0 h). Then, the thawed semen was incubated at 37ºC in a water bath and the sperm motility was recorded at 1h interval up to 3h to assess the survivability of frozen-thawed sperms.

The hypo-osmotic swelling test is based on the permeability of intact sperm cell plasma membrane, which causes spermatozoa to "swell" under hypo-osmotic conditions, when an influx of water results in an expansion of sperm cell volume. A minimum of 200 spermatozoa per slide were studied under phase contrast microscope (40X) for typical "tail curling" indicative of hypo-osmotic reaction. The proportion of hypo-osmotic reacted spermatozoa was calculated as the number of structurally altered spermatozoa, divided by total number of spermatozoa counted and multiplied by 100. The frozen semen should contain at least 40% hypo-osmotic reacted spermatozoa.

Acrosome is a cap like structure and covers 60% of anterior portion of the spermatozoa head. The status of acrosome integrity could be used as a suitable marker for fertilizing ability of spermatozoa after cryopreservation. Giemsa-stained spermatozoa per slide were examined for acrosome integrity under phase contrast microscope. The frozen semen should contain at least 70% spermatozoa with intact acrosome to ensure better fertilization.

RESULTS AND DISCUSSION

The post-thaw motility and post-thaw incubation survival were influenced ($p<0.05$) by ejaculate, period and season (Table 1). The farm had no effect ($p>0.05$) on post-thaw motility and post-thaw incubation survival (Table 1). The winter and northeast monsoon season had no effect ($p>0.05$) on post-thaw motility and post-thaw incubation survival when compared to summer. In summer season, the post-thaw motility was lowest (41.51%) and even the post-thaw incubation survival of sperms was very poor (Table 1). This suggested that good summer management of breeding bulls to overcome heat stress is necessary to maintain the quality of frozen semen straws. The frozen semen doses produced from the second ejaculate had a better post-thaw incubation survival when compared to the first ejaculate ($p<0.05$, Table 1). The reduction in post-thaw motility at successive time intervals could be due to increased oxidative metabolism and increased toxicity of the medium due to peroxide formation with an increase in number of dead spermatozoa in Tharparkar bulls (Kedia *et al*., 2013).

The period of semen collection had an impact ($p<0.05$) on hypo-osmotic swelling of spermatozoa, while other non-genetic factors viz. farm, ejaculate and season had no effect ($p>0.05$, Table 1). Among the periods, period III had higher per cent of hypo-osmotic swelling than the other periods ($p<0.05$, Table 1). Earlier workers had reported lower values for hypo-osmotic swelling of spermatozoa in crossbred Holstein Friesian bulls (Mandal *et al*., 2007; Kumar and Srivastava, 2008). All the frozen semen samples tested for this trait in the present study had >40% swollen spermatozoa under hypo-osmotic conditions in crossbred Holstein Friesian bulls.

The ejaculate and period had effect ($p<0.05$), but farm and season had no effect ($p>0.05$) on percent acrosome integrity (Table 1). Period I had higher

Table 1: Impact of non-genetic factors on post-thaw motility and post-thaw incubation survival of frozen semen samples (n=65) of crossbred Holstein Friesian bulls (n=14)

Factor	Post-thaw motility and Post-thaw incubation survival, %				Hypo-osmotic swelling, %	Acrosome integrity, %
	0 h	1 h	2 h	3 h		
Farm	NS				NS	NS
I	51.4±0.3 (24)	44.9±0.7 (24)	27.9±1.2 (24)	16.3±2.4 (24)	74.9±0.0 (24)	80.6±0.0 (24)
II	48.6±0.0 (41)	35.6±0.0 (41)	22.7±0.1 (41)	11.2±0.0 (41)	55.9±0.1 (41)	80.9±0.1 (41)
Ejaculate	*				NS	*
I	46.9±0.0 (21)	34.6±0.0 (21)	18.6±0.2 (21)	8.92±0.3 (21)	57.4±0.1 (21)	80.4±0.2 (21)
II	48.7±0.0 (44)	39.6±0.0 (44)	22.4±0.2 (44)	14.2±0.3 (44)	58.8±0.1 (44)	85.9±0.2 (44)
Period	*				*	*
I (2007-08)	48.6±0.0^{b} (12)	33.6±0.0^{b} (12)	21.6±0.0^{b} (12)	12.9±0.0^{a} (12)	52.5±1.4^{b} (7)	81.6±0.2^{a} (7)
II (2009-10)	46.6±0.0^{c} (28)	32.7±0.0^{c} (28)	16.4±0.1^{d} (28)	6.00±0.2^{c} (28)	47.2±0.5^{c} (18)	81.5±0.2^{a} (18)
III (2011-12)	46.9±0.0^{c} (17)	29.2±0.2^{d} (17)	19.4±0.4^{c} (17)	8.02±0.6^{b} (17)	57.9±0.3^{a} (26)	78.4±0.4^{b} (26)
IV (2013-14)	49.3±0.3^{a} (8)	38.7±0.8^{a} (8)	24.9±1.5^{a} (8)	12.1±2.4^{a} (8)	46.9±0.8^{d} (14)	77.0±0.2^{b} (14)
Season	*				NS	NS
Winter	51.9±0.0^{a} (28)	42.9±0.1^{a} (28)	25.5±0.2^{a} (28)	17.4±0.4^{a} (28)	59.5±0.2 (21)	82.4±0.3 (21)
Summer	41.5±0.0^{b} (27)	27.8±0.1^{b} (27)	12.2±0.3^{b} (27)	3.04±0.5^{b} (27)	57.4±0.2 (23)	86.5±0.3 (23)
NE monsoon	50.2±0.0^{a} (10)	41.1±0.0^{a} (10)	24.9±0.1^{a} (10)	17.4±0.2^{a} (10)	57.4±0.0 (21)	80.7±0.1 (21)

*p<0.05; p<0.05 - Means with different superscript within classes differ significantly; Figures in parentheses indicate number of observations; NE - Northeast

acrosome integrity, which was not different from period II (p>0.05, Table 1). The acrosome integrity estimated in the present study is much higher than an earlier report in crossbred Holstein Friesian bulls (Kumar and Srivastava, 2008). All the frozen semen samples tested had acrosome integrity >70% which qualified them for artificial insemination use.

Overall, the frozen semen quality assurance tests are mandatory to assess the quality of frozen semen straws produced in every frozen semen stations. During freezing-thawing process, the spermatozoa were subjected to physical stress which could lead to reduced motility. Poor freezability is one of the major disposals of crossbred bulls in frozen semen stations (Mukhopadhyay *et al*., 2010; Vijetha *et al*., 2014). In order to identify the poor freezability, these tests could help to ascertain the genetic merit of each bull to the process of cryopreservation.

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