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Non-genetic effects and repeatability estimates of post-thaw evaluation traits in Murrah buffalo bulls

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Abstract
The present study aims to determine the effect of non-genetic factors on post-thaw evaluation traits and estimate the traits repeatability and correlation coefficients in Murrah buffalo bulls. The data on post-thaw incubation survival (n=1745), hypo-osmotic swelling (n=1745), and acrosome integrity (n=1402) collected from 73 bulls maintained during the period 2005 to 2015 were used for analyses. Single trait univariate analyses under General Linear Model were performed to study the non-genetic effects. The repeatability was estimated by Restricted Maximum Likelihood Method (REML) using WOMBAT programme while the correlation coefficients were calculated using Pearson's correlation coefficient. The overall least-squares means for post-thaw incubation survival (at four-time intervals), hypo-osmotic swelling, and acrosome integrity were 52.42% at 0 h, 45.44% at ½ h, 37.87% at 1h and 29.82% 1½ h; 61.71% and 69.81%, respectively. Similarly, the repeatability estimates were 0.03 at 0 h, 0.02 at ½ h, 0.03 at 1 h, and 0.03 at 1½ h; 0.44 and 0.64, respectively. The ejaculate number, period, season, and age showed a significant effect (P<0.01) on all traits, besides the non-significant effect of ejaculate on acrosome integrity and age on hypo-osmotic swelling. The performance of traits was relatively better in the second ejaculate and bulls in the age group of 68 months and above. However, a significant minimal variation was observed among the periods and seasons. The correlation coefficients were highly significant (P<0.01) and positive for all traits except the negative correlation between hypo-osmotic swelling and acrosome integrity. Thus, this would be helpful to determine the breeding efficiency of the bulls and plan suitable improvisation strategies in the semen station.

Keywords: Post-thaw evaluation traits, repeatability, Murrah bulls

Introduction
Murrah buffaloes are the most commonly used breed for genetic up-grading of non-descript buffaloes and crossbreeding with local breeds to increase milk and meat production. Artificial insemination with frozen semen has proved to be the best tool worldwide for achieving this genetic improvement using the germplasm of superior quality bulls. Although semen production parameters help to assess the quality of semen and determine the extension rate and frozen semen doses for semen samples, the suitability of semen for artificial insemination largely depends on the freezability of semen. Frozen semen evaluation is a necessary procedure in any semen production laboratory since its quality determines the conception rate and reflects the quality of the bulls used for frozen semen production. The evaluation of frozen semen is done to assess spermatozoa's viability and morphological integrity before using it for artificial insemination. Since a considerable number of spermatozoa will be rendered non-motile by freezing and thawing, the assessment of sperm for its structural and functional integrity is of utmost importance. Hence, post-thaw motility, post-thaw incubation survival, hypo-osmotic swelling, and acrosome integrity are considered as frozen semen evaluation traits since these traits influence spermatozoa's fertilizing ability. Post-thaw motility and post-thaw incubation survival are the major traits that qualify the frozen semen for artificial insemination. Acrosome integrity and Hypo-osmotic swelling (HOS) test evaluate the integrity of acrosome and plasma membrane whether the cell membrane is biochemically active; these play a significant role in determining the quality of the frozen semen used for artificial insemination. Information regarding the role of environmental factors, repeatability estimates, and phenotypic correlation of frozen semen evaluation parameters in Murrah buffaloes is very minimal. Hence, the study was carried out to evaluate the effects of these components on post-thaw evaluation traits of frozen semen in Murrah buffalo bulls.

Materials and Methods

Data on frozen semen evaluation traits such as post-thaw incubation survival, hypo-osmotic swelling, and acrosome integrity were collected from various laboratory registers maintained in Exotic Cattle Breeding Farm, where Murrah buffalo bulls are exclusively maintained for frozen semen production. These traits were evaluated once in three months for all the breeding bulls involved in semen production. The post-thaw incubation survival was evaluated by recording the motility of freeze-thawed semen incubated at 37ºC in a water bath every half an hour for a minimum period of one and half hours to assess the sustainability of motility after freezing. A minimum of 10 to 15% reduction in motility after one hour was acceptable.

The hypo-osmotic swelling test was performed as per the procedure described by Jeyendran *et al.* (1984) [6] to ascertain the sperm plasma membrane's functional integrity. A hypo-osmotic solution containing equal parts of fructose and sodium citrate was prepared with 150 mOsm/L osmolality. One ml of hypo-osmotic solution was mixed with 0.1 ml of thawed semen and incubated at 37ºC for 30 minutes. The smears were prepared and stained with 3% Rose Bengal for 10 minutes and examined under phase contrast microscope for typical "tail curling" spermatozoa indicative of hypo-osmotic reaction. The percent hypo-osmotic reacted spermatozoa were calculated as the number of altered spermatozoa X 100 divided by the total spermatozoa count. The frozen semen should contain at least 40% hypo-osmotic reacted spermatozoa. The acrosome integrity of spermatozoa was estimated using the Giemsa staining technique as per Hancock's standard procedure (1952) [5]. The frozen semen should contain at least 70% of intact acrosomes to ensure effective fertilization.

A total of 1745 records on post-thaw incubation survival and hypo-osmotic swelling, and 1402 records on acrosome integrity, collected from 73 Murrah bulls maintained during the period 2005 to 2015 were subjected to analyse the environmental effects, *i.e.,* ejaculate number, season, period, and age of the bulls. The year was classified into four seasons as winter (December to February), summer (March to May), south-west monsoon (June to August), and north-east monsoon (September to November). The entire duration of the study was classified into five periods: Period I (2005-2007), II (2008-2009), III (2010-2011), IV (2012-2013), and V (2014-2015). The age of the bulls was classified into six groups as 18 to 42 months (I), 43 to 67 months (II), 68 to 92 months (III), 93 to 117 months (IV), 118 to 142 months (V), and >142 months (VI).

Statistical analyses

A. The statistical model used for the analysis of post-thaw incubation survival and hypo-osmotic swelling:

$$Y_{ijklm} = \mu + E_i + S_j + P_k + A_l + e_{ijklm}$$

Where,

Y_{ijklm} = frozen semen evaluation trait of m^{th} individual observation belonging to i^{th} ejaculate, j^{th} season, k^{th} period and l^{th} age

μ = overall mean

E_i = effect of i^{th} ejaculate (i = 1, 2)

S_j = effect of j^{th} season (j = 1 to 4)

P_k = effect of k^{th} period (k = 1 to 5)

A_l = effect of l^{th} age (l = 1 to 6)

e_{ijklm} = residual random error, NID (0 and σ^2_e)

B. The statistical model used for the analysis of acrosome integrity:

$$Y_{ijklm} = \mu + E_i + P_j + S_k + A_l + e_{ijklm}$$

Where,

Y_{ijklm} = semen production trait of m^{th} individual observation belonging to i^{th} ejaculate, j^{th} period and k^{th} season and l^{th} age

μ = overall mean

E_i = effect of i^{th} ejaculate (i = 1, 2)

P_j = effect of j^{th} period (j = 1 to 4)

S_k = effect of k^{th} season (k = 1 to 4)

A_l = effect of l^{th} age (l = 1 to 6)

e_{ijklm} = residual random error, NID (0 and σ^2_e)

As the parameters are expressed in percentage, they were adjusted to the percentage angular transformation as per Snedecor and Cochran (1989) [9]. The means and standard errors are expressed in percentages with a precision of two decimals after angles reconversion.

Repeatability was estimated for all the repeatable semen production traits as an intra-class correlation between records of the same bull (Becker, 1975) [3]. The variance components were estimated by Restricted Maximum Likelihood (REML) using the WOMBAT programme (Meyer, 2007) [8].

$$Y_{ijk} = \mu + B_i + a_j + e_{ijk}$$

Where,

Y_{ijk} = the k^{th} record of i^{th} bull in the j^{th} non-genetic effect

μ = overall mean

B_i = effect of i^{th} bull (random effect)

a_j = effect of j^{th} non-genetic effect (fixed effect) 'a' maybe one or more significant non-genetic effects

e_{ijk} = random error NID (0 and σ^2_e)

Repeatability value was obtained as intra-class correlation (r)

$$r = \frac{\sigma^2_b}{\sigma^2_b + \sigma^2_e}$$

The standard error of repeatability was calculated as per Swiger *et al.* (1964) [12].

$$SE(r) = \sqrt{\frac{2(n. - 1)(1-r)^2 [1+(k_1-1) r^2]}{k_1^2(n.- N) (N-1)}}$$

Where,

N = number of bulls

n. = total number of observations

r = repeatability of the trait

k_1 = effective number of records per bull

σ^2_b = between bulls variance component

σ^2_e = error variance component

The coefficients of correlation between the frozen semen evaluation traits were estimated by Pearson's correlation coefficient (r) using SPSS (Statistical Package for Social Sciences) version 20.

Results and Discussion

The evaluation of frozen semen for its post-thaw qualities plays a significant role in assessing the vitality of spermatozoa under freezability, which in turn validates fertilizing capacity and fertility rate. The overall least-squares means for post-thaw incubation survival, hypo-osmotic swelling, and acrosome integrity and the effects of non-genetic factors such as ejaculate, period, season, and age on these parameters were presented in Tables 1 and 2. The least-squares analysis of variance for the non-genetic factors is given in Table 3.

Post-thaw incubation survival of spermatozoa: The overall least-squares mean for post-thaw incubation survival at 0, ½, 1, and 1½ hours were 52.42 ± 0.23, 45.44 ± 0.28, 37.87 ± 0.33, and 29.82 ± 0.37 percent, respectively (Table 1). The non-genetic factors such as ejaculate, period, and age of the bull exhibited a highly significant (P<0.01) effect on post-thaw incubation survival of spermatozoa, at the same time, the effect of season was significant (P<0.05). The post-thaw motility at 0, ½, 1, and 1½ hours of incubation for frozen semen straw obtained from the second ejaculate (52.71 ± 0.30, 45.83 ± 0.36, 38.29 ± 0.42 and 30.68 ± 0.48 percent, respectively) were slightly higher than the first ejaculate (52.13 ± 0.24%, 45.05 ± 0.29%, 37.45 ± 0.34%, and 28.97 ± 0.39%). Among the different periods, period II showed a higher post-thaw incubation motility of 53.80 ± 0.36%, 48.94 ± 0.44%, 42.94 ± 0.51%, and 35.47 ± 0.59% for 0, ½, 1, and 1½ hours of incubation followed by period III and V. Concerning seasons, the highest percentage of post-thaw incubation motility was seen in the winter season (53.27 ± 0.32, 45.99 ± 0.39, 38.56 ± 0.45, and 30.58 ± 0.51 percent) when compared to summer season and monsoons. The post-thaw incubation survival was better for bulls, in the age group of 68-92 months to >142 months. The minimal variation observed among the periods and seasons in this study might be due to implementation of uniform managemental practices for the bulls maintained in the semen station. The environmental condition and management practices directly impact the quality of semen used for frozen semen straw production; hence maintaining the bulls in better environmental conditions void of external stress will improve the quality of the semen. The bull's performance was better as the age advances indicating that the bulls selected for semen production are of superior quality germplasm. The bull calves are selected directly from Murrah buffalo's native breeding track based on the breeding records of sire and dam and its pedigree value. On perusal of literature, there is a shortage of literature on post-thaw incubation survival of spermatozoa in Murrah buffalo bulls to compare the present results.

Hypo-osmotic swelling of spermatozoa: The overall least-squares mean for hypo-osmotic swelling (HOS) was 61.71 ± 0.45% (Table 2). All the frozen semen samples tested for this trait showed more than 40% swollen spermatozoa under hypo-osmotic conditions. The ejaculate, period, and season expressed a highly significant (P<0.01) influence on hypo-osmotic swelling, but the bulls age did not influence HOS significantly. The second ejaculate comparatively had a higher percentage of hypo-osmotic swollen spermatozoa (62.83 ± 0.57%) than the first ejaculate (60.59 ± 0.46%). The period I recorded significantly highest hypo-osmotic swelling of 65.66 ± 0.59%, compared to other periods with the least percentage in the period V (62.49 ± 0.73, 62.29 ± 0.75, 60.98 ± 0.70, 57.14 ± 0.80 percent, respectively). Among the seasons, the summer season exhibited a higher percentage of hypo-osmotic swelling (64.04 ± 0.58%), followed by winter and north-east monsoon with a non-significant variation between these two seasons (61.56 ± 0.61% and 61.40 ± 0.63%). The variation might be due to better management practices implemented during the summer season to reduce the impact of extreme temperature and humidity. This is in agreement with the report of Singh *et al.* (2013) [11] who proved that microclimatic modification during the hot and humid periods significantly improved the performance of hypo-osmotic swelling in Murrah bulls. The least-squares mean prevailed in the present study was higher than the earlier reports of Mandal *et al.*, 2003 [7] (58.18%), Singh *et al.*, 2013 [11] (58.3%), and Bhakat *et al.*, 2015 [1] (52.72%). However, Shukla and Misra (2005) [10] recorded an overall mean of 67.35%, which is higher than the present findings and concluded that the variation might be due to live sperm count, season, and age of the bulls. The effect of season on hypo-osmotic swelling observed in the current study is in accordance with Mandal *et al.* (2003) [7] and Bhakat *et al.* (2015) [1] who reported a significant seasonal variation in semen volume, mass activity, sperm concentration per ml as well as per ejaculate and acrosome integrity. But it is concluded that semen production was optimal during winter, low during summer, and intermediate during the rainy season, which is contrary to the present findings as better result was achieved in summer on par with the cooler seasons.

Acrosome integrity of spermatozoa: The overall least-squares mean for acrosome integrity was 69.81 ± 0.33% (Table 2). The non-genetic factors, *i.e.*, period, season, and age of the bull, owned a highly significant (P<0.01) effect on acrosome integrity, but ejaculate number did not significantly influence the parameter. Acrosome integrity was maximum at north-east monsoon (70.73 ± 0.51%) followed by south-west monsoon, summer, and winter (70.62 ± 0.45%, 69.31 ± 0.43%, and 68.53 ± 0.44%) seasons but the variation between the seasons were minimal. Bulls maintained during period III to V (2010-2015) had a better performance without any significant difference; meanwhile, the lowest value was recorded in period II. Similarly, bulls in the age group of 43-67 months to >142 months displayed the maximum percentage, ranging from 68.74 to 71.89%, with the least in the age group of 18 to 42 months (67.61 ± 1.25%). Bhosrekar *et al.* (1991) [2] studied the effect of deep freezing on sperm morphology in Murrah bulls and reported mean acrosome integrity of 92.00%. Similarly, Chowdhury *et al.* (2014) [4] and Bhakat *et al.* (2015) [1] evaluated the acrosome integrity of frozen semen and recorded an overall mean of 73.74% and 70.10%, respectively. The least squares mean for acrosome integrity acquired in the current study is relatively lower than the earlier reports. With respect to non-genetic effects, Bhosrekar *et al.* (1991) [2] reported that season had a significant impact on acrosome integrity with the highest value in the autumn season followed by summer; this is inconsistent with the present findings. However, Bhakat *et al.* (2015) [1] obtained a significant seasonal effect, but a higher value was recorded in the winter

Table 1: Least-squares means (± S.E.) for post-thaw incubation survival

Effects	No. of ejaculates	Post-thaw incubation survival (%)			
		0 hour	½ hour	1 hour	1½ hour
Overall	1745	52.42 ± 0.23	45.44 ± 0.28	37.87 ± 0.33	29.82 ± 0.37
Ejaculate		**	**	**	**
I	1237	52.13 ± 0.24	45.05 ± 0.29	37.45 ± 0.34	28.97 ± 0.39
II	508	52.71 ± 0.30	45.83 ± 0.36	38.29 ± 0.42	30.68 ± 0.48
Period		**	**	**	**
I (2005 - 2007)	463	53.23^{a} ± 0.30	39.72^{e} ± 0.37	28.25^{e} ± 0.43	18.35^{c} ± 0.49
II (2008 - 2009)	404	53.80^{a} ± 0.36	48.94^{a} ± 0.44	42.94^{a} ± 0.51	35.47^{a} ± 0.59
III (2010 - 2011)	335	51.56^{c} ± 0.39	47.16^{b} ± 0.47	41.67^{b} ± 0.54	35.08^{a} ± 0.62
IV (2012 -2013)	302	51.31^{c} ± 0.38	44.67^{d} ± 0.47	37.58^{d} ± 0.53	30.15^{b} ± 0.61
V (2014 - 2015)	241	52.23^{b} ± 0.41	46.73^{c} ± 0.51	38.91^{c} ± 0.58	30.08^{b} ± 0.67
Season		*	*	*	*
Winter	421	53.27^{a} ± 0.32	45.99^{a} ± 0.39	38.56^{a} ± 0.45	30.58^{a} ± 0.51
Summer	551	52.91^{b} ± 0.30	45.90^{a} ± 0.37	37.66^{b} ± 0.42	29.26^{c} ± 0.48
South-west monsoon	380	51.93^{c} ± 0.33	44.95^{b} ± 0.41	37.50^{c} ± 0.47	29.30^{c} ± 0.54
North-east monsoon	393	51.58^{d} ± 0.33	44.93^{b} ± 0.40	37.75^{b} ± 0.46	30.16^{b} ± 0.53
Age		**	**	**	**
I (18 - 42 m)	248	51.83^{d} ± 0.38	43.68^{e} ± 0.46	35.91^{e} ± 0.53	28.08^{d} ± 0.61
II (43 - 67 m)	557	51.72^{e} ± 0.26	44.29^{d} ± 0.32	36.50^{d} ± 0.36	28.25^{d} ± 0.42
III (68 - 92 m)	375	52.99^{a} ± 0.30	46.31^{b} ± 0.36	38.64^{b} ± 0.42	30.65^{b} ± 0.48
IV (93 - 117 m)	391	52.62^{b} ± 0.29	45.59^{c} ± 0.35	37.82^{c} ± 0.40	30.07^{b} ± 0.46
V (118 - 142 m)	152	52.43^{c} ± 0.47	45.32^{c} ± 0.58	37.41^{c} ± 0.67	29.48^{c} ± 0.76
VI (>142 m)	22	52.94^{a} ± 1.13	47.46^{a} ± 1.38	40.94^{a} ± 1.59	32.44^{a} ± 1.82

**-Highly significant (P<0.01); *-Significant (P<0.05); Means with at least one common superscript within classes do not differ significantly.

Table 2: Least-squares means (± S.E.) for hypo-osmotic swelling and acrosome integrity

Effects	Hypo-osmotic swelling (%)	Acrosome integrity (%)
Overall	61.71 ± 0.45 (1745)	69.81 ± 0.33 (1402)
Ejaculate	**	NS
I	60.59 ± 0.46 (1237)	69.72 ± 0.35 (1026)
II	62.83 ± 0.57 (508)	69.90 ± 0.40 (376)
Period	**	**
I (2005 - 2007)	65.66^{a} ± 0.59 (463)	-
II (2008 - 2009)	60.98^{c} ± 0.70 (404)	60.52^{b} ± 0.44 (519)
III (2010 - 2011)	62.29^{b} ± 0.75 (335)	73.31^{a} ± 0.80 (335)
IV (2012 -2013)	62.49^{b} ± 0.73 (302)	73.42^{a} ± 0.52 (307)
V (2014 - 2015)	57.14^{d} ± 0.80 (241)	72.55^{a} ± 0.78 (241)
Season	**	**
Winter	61.56^{b} ± 0.61 (421)	68.53^{c} ± 0.44 (381)
Summer	64.04^{a} ± 0.58 (551)	69.31^{b} ± 0.43 (403)
South-west monsoon	59.85^{c} ± 0.64 (380)	70.62^{b} ± 0.45 (348)
North-east monsoon	61.40^{b} ± 0.63 (393)	70.73^{a} ± 0.51 (270)
Age	NS	**
I (18 - 42 m)	60.66 ± 0.73 (248)	67.61^{b} ± 1.25 (163)
II (43 - 67 m)	60.79 ± 0.50 (557)	70.43^{a} ± 0.51 (488)
III (68 - 92 m)	61.35 ± 0.57 (375)	71.68^{a} ± 0.51 (325)
IV (93 - 117 m)	62.02 ± 0.56 (391)	68.74^{a} ± 0.46 (277)
V(118 - 142 m)	61.04 ± 0.92 (152)	69.91^{a} ± 0.88 (146)
VI (>142 m)	64.41 ± 2.18 (22)	71.89^{a} ± 3.35 (3)

**-Highly significant (P<0.01); NS-Non-significant; Means with at least one common superscript within classes do not differ significantly; Figures in parentheses
indicate the number of observations.

Table 3: Least-squares analysis of variance for factors affecting frozen semen evaluation traits

Source of variation	Df	Post-thaw incubation survival	Hypo-osmotic swelling	Acrosome integrity
		MSS	MSS	MSS
Ejaculate	1	1365.19**	1789.61**	8.80^{NS}
Period	4	21273.12**	2358.1**	317.96**
Season	3	432.78*	1380.01**	5226.82**
Age	5	913.78**	113.64^{NS}	137.64**
Error	1731	154.72	100.10	33.715

**-Highly significant (P<0.01); NS-Non-significant; MSS-Mean sum of squares

Season followed by the rainy and summer season, which is not concurrent with the present study as the performance was optimal in all the seasons with minimal variation.

The repeatability estimates of frozen semen evaluation traits are represented in Table 4. The repeatability measures for the frozen semen evaluation traits were low to high, ranging from 0.02 to 0.64. The post-thaw incubation survival was the least repeatable trait (0.02), whereas the highest repeatability was observed in acrosome integrity (0.64). Literature information on repeatability estimates of post-thaw evaluation traits in Murrah buffaloes is very scanty. Taraphder *et al.* (2001) [13] studied the repeatability estimate of semen characteristics with 110 semen ejaculates collected from 22 Murrah bulls. They reported repeatability estimates of 0.104 and 0.135 for post-thaw motility at 0 and 1 hour of incubation and 0.155 for acrosome integrity. In comparison with the earlier report, higher repeatability estimates for acrosome integrity (0.64) and lower estimates for post-thaw incubation survival at 0 (0.03) and 1 (0.03) hour was noticed in the present study. Repeatability is the measure of the correlation between the repeated records of the same animal and has practical application in predicting future performance. Since repeatability is considered as an upper limit to heritability, it gives an idea about the heritability when there are no pedigree records available for the traits. Hence, the selection of bulls based on repeatability would be more useful because the bull calves are purchased from different sources, and grouping of progenies per sire is almost impossible to estimate the heritability of the frozen semen evaluation traits. The lower repeatability measure for post-thaw incubation survival indicates that the trait was influenced more by the non-genetic factors rather than heritable causes. On the other hand, moderate to high repeatability estimates were obtained for hypo-osmotic swelling and acrosome integrity, indicating that the performance of the bull for these traits was consistent in nature for repeated evaluation with minimal influence by environmental factors. Therefore, this would be useful to evaluate the breeding soundness of the bulls and select the bulls relatively at an early stage of semen production based on a few initial records for these traits.

The coefficient of correlation between the frozen semen evaluations traits are presented in Table 5. Although the post-thaw incubation survival at all incubation intervals revealed a highly significant (P<0.01) and positive correlation with hypo-osmotic swelling and acrosome integrity, but it is low to moderate from 0.16-0.29 and 0.19-0.36, respectively. However, the correlation coefficients between different incubation intervals were high (0.42-0.92) and significant (P<0.01) in a positive direction. Hypo-osmotic swelling had a highly significant (P<0.01) negative correlation with acrosome integrity (-0.19), featuring that these two parameters do vary independently of each other. Since phenotypic correlation is a measure of the strength of the relationship between the performance of one trait and another trait. The estimation of phenotypic correlations between the frozen semen evaluation traits would help to understand the underlying relationship between these parameters. The present findings on the positive correlation of hypo-osmatic swelling and acrosome integrity with post-thaw incubation survival indicate that the frozen semen with more of the number of viable spermatozoa has a direct impact on these traits, as live spermatozoa with the active functional plasma membrane and acrosome are prime requisite to evaluate these traits. This is in accordance with Shukla and Misra (2005) [10] who revealed that there was a significantly positive correlation between initial motility and sperm concentration (0.18) and with HOST (0.43), while HOST had a positive correlation of 0.92 with the number of live sperm in semen. There is a lack of details available on correlation coefficients of frozen semen evaluation parameters in Murrah bulls; hence it could not be compared with the earlier reports.

Table 4: Repeatability estimates of post-thaw evaluation traits in Murrah buffalo bulls

Traits		No. of records	Residual variance ± S.E. (σ^2e)	Repeatability ± S.E.
Hypo-osmotic swelling		1745	0.96 ± 0.01	0.44 ± 0.02
Acrosome integrity		1402	0.36 ± 0.05	0.64 ± 0.05
Post-thaw incubation survival	0 hr	1745	0.97 ± 0.01	0.03 ± 0.01
	½ hr		0.98 ± 0.01	0.02 ± 0.01
	1 hr		0.98 ± 0.01	0.03 ± 0.01
	1½ hrs		0.97 ± 0.01	0.03 ± 0.01

Table 5: Correlation between frozen semen evaluation traits in Murrah buffalo bulls

Traits		Hypo-osmotic swelling	Acrosome integrity	Post-thaw incubation survival		
				0 h	½ h	1 h
Acrosome integrity		-0.19**				
Post-thaw incubation survival	0 h	0.29**	0.21**			
	½ h	0.17**	0.19**	0.71**		
	1 h	0.16**	0.32**	0.52**	0.89**	
	1½ h	0.19**	0.36**	0.42**	0.79**	0.92**

****-**Highly significantly (P<0.01)

Conclusion

The non-genetic factors significantly influenced the frozen semen evaluation traits with better performance in second ejaculate and bulls at 68 months and above. The minimal variations noticed in the present investigation between the periods and seasons might be due to sound environmental management and feeding practices. Since repeatability estimates were low to high, there is a scope for selecting bulls at an early stage based on the performance of highly repeatable traits. Therefore, evaluation of frozen semen for its genetic and non-genetic components would help assess the performance of the bulls in quality semen production and execute suitable management practices in the semen station for improvement.

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