RESEARCH ARTICLE

Physico-Morphological Attributes, Freezability, Fertility and their Correlations in Jaffarabadi Buffalo Bull Semen

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Abstract

The present study was carried out on semen of six Jaffarabadi buffalo bulls, aged 6-8 years, under twice weekly semen collection schedule. In all 36 semen ejaculates (6/bull) were evaluated for routine physical characteristics including sperm quality and oxidative stress parameters at pre-freeze and post-thaw stages of cryopreservation and fertility using different Sericin concentrations in AndroMed® extender. All bulls donated milky-white semen with identical mean mass motility score, while ejaculate volume and sperm concentration differed significantly among bulls. The values of sperm progressive motility (%), viability (%), abnormality (%), HOST reactivity (%), acrosome integrity (%), lipid peroxidation (MDA µM) and total antioxidant capacity (TAC µM) of pre-freeze (on dilution) and post-thawed semen averaged 84.24 ±0.24 & 53.42±0.32, 82.43±0.26 & 57.38±0.49, 6.14±0.12 & 13.56±0.22, 72.84±0.31 & 45.65±0.48, 81.56±0.35 & 49.73 ±0.52, 7.71±0.04 & 8.35±0.05, and 315.80±5.99 & 292.89±7.40, respectively. The overall first service conception rate achieved was 36.73 (551/1500) %, and it varied from 28.00 to 44.66% between levels of Sericin. Pearson's correlations amongst the pre-freeze as well as post-thawed semen parameters, and of pre-freeze with post-thawed semen parameters including CRs of frozen-thawed semen worked out using bull-wise and additive-wise 30 mean values revealed significant positive associations among sperm motility, viability, acrosome integrity, HOST reactive sperm, total antioxidant capacity (TAC), and first service CR, and all these had negative correlations with abnormal sperm and the lipid peroxidation (MDA) status in both pre-freeze and post-thawed semen. Further, the post-thawed sperm quality and fertility could be predicted well with highly significant positive correlations of pre-freeze sperm quality parameters and TAC in Jaffarabadi buffalo bulls.

Key words: Additive sericin, Cryopreservability, Fertility, Jaffarabadi buffalo semen, Pearson's correlations.

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Introduction

In order to use AI widely and improve the genetics of buffalo, sperm must be stored for a longer period. However, buffalo spermatozoa are less freezable (Andrabi, 2009) and the conception rate with frozen-thawed semen in field conditions is lower (30%) than that of cattle (Anzar *et al.,* 2003). Cryopreservation is reported to reduce buffalo bull sperm quality including DNA integrity for greater generation of reactive oxygen species (ROS), resulting in lipid peroxidation of the plasma membrane and subsequently spermatozoa death (Kadirvel *et al.,* 2009). The ROS can attack phospholipids, which are crucial for the structural integrity and function of the plasma membrane. Bull semen motility, plasmalemma integrity, and fertility are all inversely linked with lipid peroxidation level (Kasimanickam *et al.,* 2007).

Naturally occurring antioxidants in semen protect the sperm from damage caused by ROS in the environment (Bilodeau *et al.,* 2000; Andrabi, 2009). Cryopreservation and thawing, however, decrease the amount of natural antioxidants in bull semen by diluting the semen with an extender and producing an excessive amount of ROS molecules (Andrabi, 2009; Kumar *et al.,* 2011). Hence, it is recommended to incorporate antioxidants such as mifepristone, taurine, trehalose, sericin etc. into extenders,

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as they mitigate the damage inflicted on spermatozoa by cryo-treatment and improve post-thaw sperm quality and fertility (Dorji *et al.,* 2015; Patel *et al.,* 2019; Dhami *et al.,* 2020; Chaturvedi *et al.,* 2023; Vijyeta *et al.,* 2024). Moreover, there is positive association between sperm quality parameters including oxidative markers and fertility of cryopreserved bovine semen, and that fertility of cryopreserved semen can be predicted based on only a few vital sperm quality parameters of fresh and frozen semen rather than going for a plethora of time consuming tests with no additional advantage (Rao *et al.,* 2011; Lodhi *et al.,* 2008; Patel *et al.,* 2012; Patel *et al*. 2019). Hence the present study was aimed to evaluate the physico-morphological attributes, freezability and fertility using Sericin as extender additive and their interrelationships in Jaffarabadi buffalo bull semen.

Materials and Methiods

The present investigation was undertaken at Semen Freezing Laboratory, Cattle Breeding Farm, Junagadh (India) for a period of 1 year. Total six Jaffarabadi bulls, aged 6-8 years, were used and semen was collected using artificial vagina method twice weekly from each bull. The semen samples (n=36, 6/bull) were evaluated for physical characteristics and oxidative stress parameters at pre-freeze and post-thaw stages of cryopreservation including fertility response using split-ejaculate technique for different sericin concentrations in AndroMed® extender.

For this, each ejaculate was divided into five equal fractions and diluted to a final concentration of 80 million sperm/mL using AndroMed® supplemented with different concentrations of Sericin, filled and sealed in French medium straws by automatic filling and sealing machine (MRS1 Dual, IMV, France), cooled to and equilibrated for 4 h in cold handling cabinet. Straws were then frozen in LN_2 vapour for a period of 7-8 min at -140 °C on a rack, in the wide mouth cryovessel and finally plunged into LN_2 (-196 °C). Thawing of the straws was done next day in water bath at 37 °C for 30 sec. The pre-freeze and post-thaw semen parameters, *viz.,* sperm motility, viability, morphology, plasma membrane integrity (HOST), and acrosome integrity including lipid peroxidation (MDA production) and total antioxidant capacity (TAC) were evaluated in all five aliquots. The representative frozen semen straws of each combination (total 1500) preserved in LN_2 were utilized for insemination of estrus buffaloes on the farm and in field by experienced AI technicians to assess the first service conception rate (CR).

Pearson's correlations were worked out using bull- and additive-wise 30 mean values amongst the pre-freeze as well as post-thawed sperm quality and oxidative parameters, and of pre-freeze with post-thawed semen parameters including CRs of frozen-thawed semen.

Result and Discussion

Physical Attributes of Neat Semen

The mean (± SE) values of neat semen parameters like semen colour, ejaculate volume (mL), sperm concentration (million/mL) and mass activity (0-5 scale) of the Jaffarabadi buffalo bulls are depicted in Table 1.

The colour of semen in Jaffarabadi bull ranged from milky white to creamy white with the overall average score of 3.33±0.19, and there was no significant difference between bulls. Chavda *et al.* (2021) recorded predominantly milky white semen, followed by creamy white in Jaffarabadi bulls. The appearance of semen in buffalo bulls provides valuable insights into the variability and characteristics of semen in different breeds. The colour of buffalo bull semen is influenced by several factors, including the number of sperm present, sperm concentration, and viscosity, apart from contamination and genital health.

The average semen ejaculate volume in Jaffarabadi bulls under study was 5.59±0.59 mL, with a significant ($p<0.05$) difference between bulls (Table 1). Similar were the observations of Ghodasara *et al.* (2016) and Chavda *et al.* (2021) in Jaffarabadi bulls. However comparatively lower ejaculate volume has been reported in Nili-Ravi (Javed *et al.,* 2000; Ahmed *et al.,* 2018), Murrah (Bhakat *et al.,* 2011; Saini *et al.,* 2017), and Surti buffalo bulls (Chaudhary *et al.,* 2017). Difference in the semen volume in various breeds of buffaloes might be due to differences in genetics, scrotal size and weight, age and reproductive health status of bulls (Bhakat *et al.,* 2015), method and frequency of collection, nutrition, season, and managemental conditions (Bhakat *et al.,* 2011). Variations can also be due to skill of semen collector/ attendant and temperature of AV (Javed *et al.,* 2000; Ghodasara *et al.,* 2016).

The overall average sperm concentration in Jaffarabadi bulls was 1322.95±116.20 million/mL, which did not vary significantly between bulls (Table 1). This was in line with observation of Javed *et al.* (2000) in Nili-Ravi bulls, and Bhakat *et al.* (2015) and Saini *et al.* (2017) in Murrah buffalo bulls, while higher sperm concentration has been recorded in Nili-Ravi buffalo bulls (2335 M/mL) by Ahmed *et al.* (2018) and lower one in Jaffarabadi buffalo bulls (838 M/mL) by Ghodasara *et al.* (2016). Variation in sperm concentration is also attributed to different factors mentioned above.

The mean mass activity score in Jaffarabadi bulls' neat semen was 4.00±0.00, which was statistically at par in all the bulls (Table 1). The lower values of mass activity score than that observed in our study were reported by Javed *et al.* (2000) in Nili-Ravi buffalo; Bhakat *et al.,* (2011, 2015) in Murrah buffalo bulls; Chavda *et al.* (2021) in Jaffarabadi bulls. The reason for non-significant ($p<0.05$) variation between bulls and replicates may be due to the use of bulls of known mass activity, same breed and identical management conditions and frequency of semen collection.

Table 1: Physical attributes of neat semen of Jaffarabadi bulls (Mean ± SE)

Bull	Colour score (1-4)	Ejaculate volume (mL)	Concentration of sperm (M/mL)	Mass activity score (0-5)
B1	3.66±0.21	4.86±0.78^{ab}	1625.66±92.37^{b}	4.00±0.00
B2	3.33±0.21	7.90±1.36^{c}	1361.66±162.71^{ab}	4.00±0.00
B3	3.50±0.22	4.28±0.22^{a}	1444.16±67.19^{ab}	4.00±0.00
B4	3.16±0.17	5.11±0.56^{b}	1222.21±175.88^{a}	4.00±0.00
B5	3.16±0.17	4.53±0.31^{a}	1170.15±109.46^{a}	4.00±0.00
B6	3.16±0.17	6.83±0.31^{bc}	1113.83±89.58^{a}	4.00±0.00
Overall	**3.33±0.19**	**5.59±0.59**	**1322.95±116.20**	**4.00±0.00**

Means with different superscripts within column differ significantly at p<0.05 level.

Cryopreservability and Fertility of Jaffarabadi Bull Semen

The overall pre-freeze and post-thawed values of sperm quality parameters and oxidative markers, irrespective of supplementation of Sericin concentration in extender, are presented in Table 2. The overall first service conception rate achieved using 1500 AIs on farm and field in Jaffarabadi buffaloes was 36.73 (551/1500) %, and it varied from 28.00 to 44.66% between levels of Sericin. All these observations concurred well with several earlier publications already discussed at length in a separate publication with respect to effect of different levels of Sericin in extender (Vijyeta *et al.,* 2024).

Correlations (r) among Pre-Freeze Semen Parameters including CR

The correlation analysis ('r') of pre-freeze semen parameters (Table 3) revealed a highly significant (p<0.01) positive correlations of sperm motility with live sperm (0.523), intact acrosome (0.576), HOS test (0.473), and the conception rate of frozen-thawed semen (0.527). This indicates that higher individual motility in pre-freeze semen is strongly associated with sperm viability and membrane integrity and thereby the conception rates. Conversely, individual motility exhibited a highly significant (p<0.01) negative correlation with abnormal sperm (-0.504) and lipid peroxidation (-0.456), suggesting that higher sperm motility is linked to fewer abnormalities in sperm morphology and that lower lipid peroxidation levels may contribute to enhanced individual sperm motility.

Live sperm in pre-freeze semen demonstrated highly significant (p<0.01) positive correlations with intact acrosome (0.965), HOS test (0.942), TAC (0.658), and the conception rate (0.714). These findings highlight that higher levels of live sperm before freezing are strongly associated with improved sperm quality and increased post-thaw conception rates. Interestingly, live sperm exhibited a non-significant negative correlation with lipid peroxidation (-0.227), suggesting that lipid peroxidation levels may not have a significant impact on live sperm count in pre-freeze semen. Abnormal sperm in pre-freeze semen exhibited highly significant (p<0.01) negative correlations with intact acrosome (-0.781), HOS test (-0.707), total antioxidant capacity (-0.556), and the conception rate of post-thaw semen (-0.624). These results indicate that a higher percentage of abnormal sperm in pre-freeze semen is strongly associated with reduced sperm quality and decreased post-thaw conception rates.

Intact acrosome in pre-freeze semen exhibited highly significant (p<0.01) positive correlations with HOS test (0.948), TAC (0.706), and the conception rate (0.755). These results indicate that a higher percentage of sperm with intact acrosome in pre-freeze semen is strongly associated with improved sperm quality and post-thaw conception rates. HOS test in pre-freeze semen demonstrated highly significant (p<0.01) positive correlations with TAC (0.694) and the conception rate (0.713), highlighting the importance of HOST results in predicting post-thaw semen quality and conception rates.

Total antioxidant capacity of pre-freeze seminal plasma showed a highly significant (p<0.01) positive correlation with the conception rate (0.712), emphasizing the role of antioxidants in improving post-thaw semen quality and fertility outcomes. Lipid peroxidation exhibited a non-significant negative correlation with the conception rate of

Table 2: Overall mean (±SE) sperm quality and oxidative parameters of Jaffarabadi buffalo bull semen before and after cryopreservation in AndroMed extender irrespective of additive

Stage of freezing	Progressive sperm motility(%)	Lives sperm (%)	Abnormal sperm (%)	HOST reactive sperm(%)	Acrosome integrity (%)	Total antioxidant capacity(µM)	Lipid peroxidation (MDA, µM)
Pre-freeze	84.24 ±0.24	82.43 ±0.26	6.144 ±0.12	72.84 ±0.31	81.56 ±0.35	315.80 ±5.99	7.71 ±0.04
Post-thaw	53.42 ±0.32	57.38 ±0.49	13.56 ±0.22	45.65 ±0.48	49.73 ±0.52	292.89 ±7.40	8.35 ±0.05

post-thaw semen (-0.279), suggesting that lipid peroxidation may not significantly impact post-thaw conception rates.

Correlations (r) among Post-Thawed Semen Parameters including CR

The correlation analysis of post-thaw semen (Table 3) unveil significant insights into the dynamics that underlie the quality and fertility potential of post-thaw semen. The data revealed a highly significant ($p<0.01$) positive correlation between post-thaw sperm motility and the conception rate (0.841). Post-thawed live sperm exhibited highly significant ($p<0.01$) positive correlations with both post-thaw sperm motility and conception rate (0.766, 0.706). These findings emphasize the pivotal role of live sperm in influencing both post-thaw semen quality and the potential for successful conception. Conversely, the post-thawed abnormal sperm demonstrated highly significant ($p<0.01$) negative correlations with post-thaw motility and live sperm and the conception rate and (-0.900, -0.933, and -0.811, respectively). This underscores that a higher percentage of abnormal sperm in post-thaw semen is strongly associated with diminished semen quality and a reduced likelihood of achieving conception.

The post-thawed intact acrosome displayed highly significant ($p<0.01$) positive correlations with the conception rate of frozen-thawed semen, post-thaw sperm motility, and live sperm (0.863, 0.971, and 0.817, respectively), and simultaneously exhibited a highly significant ($p<0.01$) negative correlation with abnormal sperm (-0.943). These findings underscore the fundamental role of an intact acrosome in influencing not only post-thaw semen quality, but also the potential for successful conception. The HOS test of post-thawed semen revealed highly significant ($p<0.01$) positive correlations with the conception rate, sperm motility, live sperm, and intact acrosome, with correlation coefficients of 0.788, 0.888, 0.936, and 0.927, respectively. Simultaneously, it displayed a highly significant ($p<0.01$) negative correlation with abnormal sperm (-0.965). These correlations emphasize the critical importance of HOS test results in predicting post-thaw semen quality and, consequently, its profound impact on fertility outcomes.

Total antioxidant capacity of post-thawed seminal plasma exhibited highly significant ($p<0.01$) positive correlations with the post-thaw sperm motility, live sperm, intact acrosome, HOS test, and the conception rate, with correlation coefficients of 0.759, 0.715, 0.825, 0.788, and 0.857, respectively. Concurrently, it displayed a highly significant ($p<0.01$) negative correlation with abnormal sperm (-0.789). These findings suggest the central role of TAC in shaping post-thaw semen quality and its substantial influence on fertility potential. Lipid peroxidation in post-thawed semen showed a highly significant ($p<0.01$) positive correlation with abnormal sperm (0.845). Moreover, it had highly significant ($p<0.01$) negative correlations with the post-thaw sperm motility, live sperm, intact acrosome, HOS test, TAC, and conception rate (-0.775, -0.833, -0.829, -0.857, -0.691, and -0.773, resp, Table 3).

Table 3: Pearson's correlations (r) among the Pre-freeze and among the Post-thaw sperm quality parameters and antioxidant status with conception rate of frozen-thawed semen of Jaffarabadi buffalo bulls

Sperm parameters	Individual sperm motility	Live sperm	Abnormal sperm	Intact Acrosome	HOS test	TAC-Total antioxidant capacity	Lipid peroxidation	CR post-thaw semen
Correlations: Pre-freeze (freshly diluted) semen								
Sperm motility	1.00	0.523**	-0.504**	0.576**	0.473**	0.188	-0.456*	0.527**
Live sperm	0.766**	1.00	-0.736**	0.965**	0.942**	0.658**	-0.227	0.714**
Abnormal sperm	-0.900**	-0.933**	1.00	-0.781**	-0.707**	-0.556**	0.323	-0.624**
Intact Acrosome	0.971**	0.817**	-0.943**	1.00	0.948**	0.706**	-0.290	0.755**
HOS test	0.888**	0.936**	-0.965**	0.927**	1.00	0.694**	-0.184	0.713**
TAC	0.759**	0.715**	-0.789**	0.825**	0.788**	1.00	-0.049	0.712**
Lipid peroxidation	-0.775**	-0.833**	0.845**	-0.829**	-0.857**	-0.691**	1.00	-.279
CR post-thaw semen	0.841**	0.706**	-0.811**	0.863**	0.788**	-0.857**	-0.773**	1.00
Correlations: Post-thawed semen								

N=30, df=28, *p<0.05, **p<0.01, two tailed test. CR= First service conception rate, HOS= Hypo-osmotic swelling.

These results suggest that higher levels of lipid peroxidation may negatively impact post-thaw semen quality and overall fertility potential, as evidenced by its detrimental effects on multiple parameters.

Association between Pre-freeze and Post-Thawed Semen Parameters including CR

The correlation analysis between pre-freeze and post-thaw semen parameters (Table 4), revealed that the pre-freeze sperm motility had a highly significant ($p<0.01$) positive correlations with post-thaw motility (0.560), intact acrosome (0.519), TAC (0.371), and the conception rate (0.527). This suggests that higher individual sperm motility in pre-freeze semen is associated with improved post-thaw semen quality and increased fertility. Pre-freeze live sperm demonstrated highly significant ($p<0.01$) positive correlations with post-thaw sperm motility (0.816), intact acrosome (0.837), HOS test (0.664), TAC (0.703), and the conception rate (0.714). This underscores the critical role of live sperm in determining post-thaw semen quality and fertility outcomes. In contrast, pre-freeze live sperm exhibited highly significant ($p<0.01$) negative correlations with post-thaw abnormal sperm (-0.646) and lipid peroxidation (-0.642), implying that higher levels of live sperm are associated with lower occurrences of abnormal sperm and reduced lipid peroxidation.

Pre-freeze abnormal sperm displayed highly significant ($p<0.01$) positive correlations with post-thaw lipid peroxidation (0.588) and abnormal sperm (0.670), indicating that higher levels of abnormal sperms in pre-freeze semen are associated with increased lipid peroxidation and the persistence of abnormal sperm in post-thaw samples. Conversely, pre-freeze abnormal sperm showed highly significant ($p<0.01$) negative correlations with post-thaw sperm motility (-0.790), live sperm (-0.562), intact acrosome (-0.773), HOS test (-0.619), TAC (-0.642), and the conception rate of frozen-thawed semen (-0.624). These findings emphasize that a higher percentage of abnormal sperm in pre-freeze semen is linked to reduced semen quality and diminished fertility potential in the post-thaw stage.

The intact acrosome in pre-freeze semen exhibited highly significant ($p<0.01$) positive correlations with post-thaw motility (0.841), live sperm (0.497), intact acrosome (0.866), HOS test (0.695), TAC (0.757), and the conception rate (0.755). This shows the pivotal role of an intact acrosome in shaping post-thaw semen quality and fertility outcomes. Additionally, it displayed highly significant ($p<0.01$) negative correlations with post-thaw abnormal sperm (-0.701) and lipid peroxidation (-0.645), indicating that a well-preserved acrosome in pre-freeze semen is associated with fewer abnormalities and lower lipid peroxidation in post-thaw samples.

The HOS test of pre-freeze semen showed highly significant ($p<0.01$) positive correlations with post-thaw sperm motility (0.789), intact acrosome (0.810), HOS test (0.611), TAC (0.691), and the conception rate of frozen-thawed

Table 4: Pearson's correlations (r) between Pre-freeze and Post-thaw sperm quality parameters and antioxidant status including conception rate of frozen-thawed semen of Jaffarabadi buffalo bulls

Sperm parameters Pre-freeze Vs. Post-thaw		Post-thaw semen parameters							
		Sperm motility	Live sperm	Abnormal sperm	Intact Acrosome	HOS test	TAC-Total antioxidant capacity	Lipid peroxi-dation	CR post-thaw semen
Pre-freeze (On dilu-tion) semen para-meters	Individual motility	0.560**	0.160	-0.368*	0.519**	0.279	0.371*	-0.189	0.527**
	Live sperm	0.816**	0.441*	-0.646**	0.837**	0.664**	0.703**	-0.642**	0.714**
	Abnormal sperm	-0.790**	-0.562**	0.670**	-0.773**	-0.619**	-0.642**	0.588**	-0.624**
	Intact Acrosome	0.841**	0.497**	-0.701**	0.866**	0.695**	0.757**	-0.645**	0.755**
	HOS test	0.789**	0.416*	-0.634**	0.810**	0.611**	0.691**	-0.631**	0.713**
	TAC	0.742**	0.722**	-0.796**	0.798**	0.797**	0.693**	-0.762**	0.712**
	Lipid peroxidation	-0.387*	-0.302	0.386*	-0.372*	-0.385*	-0.186	0.186	-0.279

N=30, df=28, *p<0.05, **p<0.01, two tailed test.

semen (0.713). These findings highlight the significance of HOS test results in predicting post-thaw semen quality and fertility outcomes. Conversely, it exhibited highly significant ($p<0.01$) negative correlations with post-thaw abnormal sperm (-0.634) and lipid peroxidation (-0.631), indicating that higher HOS test values are associated with reduced abnormalities and lipid peroxidation in post-thaw samples.

Pre-freeze total antioxidant capacity demonstrated highly significant ($p<0.01$) positive correlations with post-thaw sperm motility (0.742), live sperm (0.722), intact acrosome (0.798), HOS test (0.797), TAC (0.693), and the conception rate (0.712). Conversely, it displayed highly significant ($p<0.01$) negative correlations with post-thaw abnormal sperm (-0.796) and lipid peroxidation (-0.762), indicating that higher TAC in pre-freeze semen is associated with reduced abnormalities and lower lipid peroxidation in post-thaw samples.

Lipid peroxidation of pre-freeze semen exhibited a positive significant ($p<0.05$) correlation with post-thaw abnormal sperm (0.386), but negative correlations with post-thaw sperm motility (-0.387), intact acrosome (-0.372), and the HOS test (-0.385). These findings suggest that higher lipid peroxidation levels in pre-freeze semen is associated with increased abnormalities and reduced motility, intact acrosome preservation, and HOS test results in post-thaw semen. Additionally, it displayed non-significant negative correlations with post-thaw live sperm (-0.302), TAC (-0.186), and the conception rate of frozen-thawed semen (-0.279), indicating that lipid peroxidation may not have a substantial impact on these parameters.

The correlation coefficients observed among physico-morphological parameters, oxidative markers, and conception rate in Jaffarabadi bull semen in this study align closely with previous research findings. Notably, these results are in line with the findings of several prior studies, including those conducted by Shelke and Dhami (2001), Lodhi *et al.* (2008), Tiwari *et al.* (2009), Mahmoud *et al.* (2013), and Patel *et al.* (2019) in the context of bovine semen. In a study conducted by Patel *et al.* (2012), significant positive inter-relationships were discovered ($p<0.01$) between individual sperm motility, viability, and the hypo-osmotic swelling test in both fresh and post-thawed semen samples. Similarly, Rao *et al.* (2011) reported a positive correlation between fertility rates upon the first insemination and parameters such as mass activity, initial motility, and sperm concentration in the semen of Ongole bulls.

Further investigations by Prasad *et al.* (1999) unveiled a positive correlation between acrosomal integrity and post-thaw livability and motility in crossbred bull semen. Shukla *et al.* (2011) found a highly significant positive correlation (0.43) between hypo-osmotic swelling (HOS) reacted sperms after dilution and after freezing of bull semen. Present findings also align with the results of Rana and Dhami (2003), Chaudhari *et al.* (2015), and Pathak *et al.* (2018). They, too, reported significant ($p<0.01$) interrelationships between various parameters such as the percentages of motile, live, abnormal sperms, intact acrosomes, and HOS reactive spermatozoa in fresh semen and post-thawed semen samples from bovine and bubaline species, with correlation coefficients ranging from 0.17 to 0.90.

Conclusion

The present study revealed that the quality of semen donated by the Jaffarabadi buffalo bulls was good to excellent and when cryopreserved in AndroMed extender yielded optimum post-thaw quality and fertility. The sperm motility, viability, HOS reactive sperm, acrosome integrity, malondialdehyde (MDA) production, total antioxidant capacity (TAC), and *in vivo* fertility could serve as potential markers for semen quality, and showed significant correlations between them. The study reveals intricate relationships between semen parameters and oxidative markers in both pre-freeze and post-thaw semen, offering valuable insights into their influence on fertility outcomes in cryopreserved Jaffarabadi bull semen. The post-thawed sperm quality and fertility could be predicted well with highly significant positive correlations of a few simple sperm quality parameters and TAC in pre-freeze and post-thawed semen of Jaffarabadi buffalo bulls.

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