Academy of Agriculture Journal

Contents lists available at www.innovativejournal.in

ACADEMY OF AGRICULTURE JOURNAL

Available online at http://innovativejournal.in/aaj/index.php/aaj

ISSN:2456-2416

Fresh and Post Thaw Quality Characteristics of Holstein Friesian Bull Semen

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Abstract: The study was conducted to determine the quality characteristics of fresh and post-thaw semen of Holstein Friesian bull. The cow bulls were maintained at Semen Production Unit, Quetta Baluchistan. The semen ejaculates were evaluated for volume, pH, mass activity, sperm concentration, sperm motility and sperm cell membrane integrity. The semen qualifying these criteria were processed for freezing. The frozen semen after thawing was assessed for progressive linear motility and percentage of intact cell membrane. The mean ejaculate volume of collected semen was recorded as 6.93, 6.59 and 6.61 ml, pH; 6.58, 6.59 and 6.61, mass activity of sperms 3.87, 3.75 and 3.5 and sperm concentration $1349.12x10^6$, $1509.37x10^6$ and $1364.37x10^6$ /ml during the month of May, June and July respectively. The color of semen was not consistent pattern, it varied as creamy white and milky white. The motility of fresh and post thaw semen was assessed as 73.9 vs 48.75%; 75 vs. 53.75%; 75 vs. 53.12% for the month of May, June and July respectively. The membrane integrity of the fresh semen samples over a period of three month was 58.37% against post-thaw membrane integrity of 44.79%. It was concluded that the objective method of Osmotic Resistance Test was found to be useful parameter for assessment of in-vitro quality of the semen. The semen of Holstein Friesian bull tolerates freezing and thawing stress and maintained reasonable good fertility. The summer season has no significant adverse effect on quality of semen and thus the semen collected could be considered for artificial insemination program.

Key words: Cattle, Holstein Friesian, Bull, Semen, Quality

Introduction:

Artificial insemination is one of the most important biological technique applied for genetic improvement of the herd. Few selected males produce enough sperms to inseminate thousands of females in a year. The number of bulls required for breeding purpose, greatly reduced and consequently the quality of bulls has become a matter of vital importance (Ax *et al.*, 2000). Artificial insemination

(AI) is now being widely used in nearly all cattle and buffaloes breeding countries. The achieving higher percentage of conception with the artificial insemination, it is essential to improve the techniques of insemination and quality of diluted semen (AWG, 2008). The fertilizing ability of spermatozoa, depends not only on the initial quality of semen, but also on the subsequent laboratory process that ends with deposition of semen in the female genital tract. The processes of semen dilution, chilling, freezing, storage, transportation and thawing for insemination invariably resulted in reduction of the viability and fertilizing capacity of spermatozoa.

The importance of the breeding program is to identify genetically superior bulls and maximize the number of offspring conceived with frozen semen through artificial insemination (AI), thus increasing the dissemination of their genes (Hallap, 2006). Although the storage of semen for 3 to 4 days may be satisfactory for day-to-day requirements of AI centers. However; it is difficult to maintain its fertility for a long time period. With the growing realization of the importance of progeny testing, it became evident that the development of new methods of semen preservation could be beneficial for existing AI industry (Dean, 2006). The fertilizing ability of spermatozoa is affected by thawing temperature and duration. The modification of temperature in freezing and thawing of semen reduces the proportion of motile spermatozoa. A sufficient number of spermatozoa may die during the process of freezing and thawing and adversely affecting the fertilizing ability of stored semen. Hence there was great need to evaluate the semen for its fertility after freezing and thawing. This study was therefore planned to investigate the effect of freezing and thawing on the quality characteristics of sperms after thawing the semen of Holstein Friesian bull.

Materials and Methods:

Experimental animals and their management

The study was conducted to evaluate the post thaw quality characteristics of Holstein Friesian bull semen. Four Holstein Friesian bulls were selected and housed at Semen Production Unit, Quetta Baluchistan. Seasonal green fodder, wheat straw, cotton seed cake and clean water were provided. Regular vaccination against foot and mouth diseases and deworming were under taken as per scheduled.

Preparation and sterilization of equipment:

All the equipment and other materials used for semen collection, evaluation and processing were cleaned with distilled water and sterilized in autoclave at temperature of 121 °C for 15 minutes at 15 pound pressure according to the procedure described by Nico-Schutte (2004).

Preparation of bulls for semen collection: The semen donor bulls were washed and cleaned before the arrival at the collection area. Ordinary water and mild brushed were used to clean the bulls. Washing of perpetual sheath with normal saline solution was done to avoid bacterial contamination of the semen. The teaser bulls were also cleaned at the back with water properly. Dry paper/towel was used after washing to remove excessive water from the area.

Semen collection: Semen was collected with artificial-vagina technique. The appropriate precautionary measures were adopted to avoid contamination of the sample. The semen was collected twice a week for the period of 12 weeks. The bulls were sexually stimulated by providing two false mount and ten to fifteen minutes sexual restraint, in between two collection.

Semen evaluation: Immediately after collection the semen samples were transferred in to the water bath and kept at 37 ^{0}C temperature for further evaluation. The semen was also determined for volume, color, and pH and sperm concentration. The mass activity, sperm motility and progressive motility percentage of spermatozoa were determined by using the phase contrast microscope. The semen concentration was measured by hemocytometer. Semen samples having a grade of ++ mass activity (out of possible +++++) and more than 60 percent progressive motility of spermatozoa were considered acceptable for further evaluation.

Assessment of fresh samples:

Appearance: Each fresh semen sample was assessed with naked eyes, any unusual things such as blood, dis-coloration, dirt, dust and debris material etc. was noted.

Color: The color of semen was judged by naked eye appearance and recorded as watery, milky white, yellowish white and creamy white etc.

Volume: Semen volume was measured directly from graduated collecting tube and recorded in ml.

PH: The pH of the semen was recorded by using digital pH meter.

Mass activity: The mass activity was evaluated in a drop of fresh undiluted semen placed on a pre-warmed slide and stage without cover-slip at low magnification (100×) and scored as into 1-4 scales.

Sperm motility: The sperm motility before freezing (fresh semen) and after thawing was determined by placing a drop of diluted semen on a clean, pre-warmed slide, covered with a cover slip and examined at magnification of 400× under light microscope. Sperm motility was scored on the basis of the percentage of spermatozoa moving straight forward direction in the field of microscope was recorded and expressed in percentage.

Sperm concentration: It was measured by Hemocytometer (fixing solution of 3% sodium chloride) as per method described by Hennery (1991).

Assessment of membrane integrity (ORT)

The fresh semen sample was used for osmotic resistance test (ORT) by the method developed by Revell and Mrode (1994) in cattle.

Extension of semen: Each semen sample was diluted with Tris based extender (Samad, 1985).

Composition of the Tris-egg yolk Extender.

Tris (hydroxymethyl-amino-methane)	3.81 g
Citric acid	1.97g
D fructose	1.25g
Egg yolk	20 ml
Glycerol	7 ml
Penicillin	1000 iu
Streptomycin	1.00 mg/ml
Distilled water	100 ml

Semen was diluted in cold cabinet by brining both the semen and extender at same temperature to prevent from cold shock. Rate of dilution was based on initial sperm concentration observed and adjusted to have 20 million spermatozoa per straw.

Equilibration time: Diluted semen was allowed to equilibrate in a cold cabinet to reach at 5 ^{0}C for 5 hrs.

Filling of straw: The filling of straws was carried out with the help of manual suction machine in 0.5-ml straw. Sealing of the open end of the straw was done by polyvinyl chloride powder.

Freezing of semen straw: Freezing of semen straw was carried out by holding the straws in liquid nitrogen vapors at 5 cm above the surface of liquid nitrogen for 5 minutes. Then the straws were plunged into liquid nitrogen (-196^{o}C). The frozen semen was stored in liquid nitrogen at least for 24 hours and then assessed for post thaw quality.

Assessment of frozen semen for post evaluation:

Thawing of semen straw: Thawing of semen straw was carried out by immersing straws in warm water at 37 ^{o}C for 15 seconds (Rasul *et al.* 2000).

Post-thaw evaluation of frozen semen: The membrane integrity of post-thaw semen was assessed by adding a drop of thawed semen in the developed by Revell and Mrode (1994) in cattle for freeze thaw semen.

Statistical analysis: Analysis of variance (ANOVA) was applied to analyze the data. The data were presented as mean and standard error of mean. Least significant difference (LSD) test was applied where appropriate.

Results:

The study was conducted on four adult Holstein Friesian bulls for the period three months. The bulls were maintained at semen production unit Quetta, Baluchistan. Twenty four semen samples were collected and utilized for this purpose. The results described below:

Macroscopic characteristics of fresh semen:

1. Semen volume: The average ejaculate volume of semen from all four bulls was recorded as 7.103 ml (Table-1). The semen volume was significantly ($P<0.05$) higher (7.75 ml) when collected in the month of July and slightly decreased (7.25 ml) in May and June. The average ejaculate semen volume of Bull 1, 2, 3 and 4 shown non-significant difference ($P>0.05$) among the bulls.

Table-1: Ejaculate volume (ml) of Holstein Friesian bull semen.

Month of semen collection	Bull-1 (ml)	Bull-2 (ml)	Bull-3 (ml)	Bull-4 (ml)	Mean (ml)
2nd. week of May	7.00	7.50	7.50	7.00	7.25 ab
4th week of May	6.00	6.50	7.00	7.00	6.62 b
2nd week of June	7.50	7.00	7.00	6.50	7.00 b
4th week of June	6.50	6.50	7.00	7.00	6.75 b
2nd week of July	8.00	7.50	6.00	7.50	7.75 a
4th week of July	7.00	7.00	7.50	7.50	7.25 ab
Mean	7.00	7.00	7.33	7.08	7.103

2. Semen pH: The mean pH values of semen samples was recorded as 6.60, collected from Holstein Friesian bulls. It was observed that the pH of semen was relatively higher (6.65) when collected in July, while a slight variation was found in semen pH when semen collected in May with semen pH of 6.62 and 6.55, respectively. The mean semen pH ejaculated from bull 1, 2, 3 and 4 was 6.58, 6.62, 6.67 and 6.53, respectively shown a non-significant (P>0.05) difference between the bulls (Table-2).

Table-2. PH of Holstein Friesian bull semen.

Month of semen collection	Bull-1 (pH)	Bull-2 (pH)	Bull-3 (pH)	Bull-4 (pH)	Mean (pH)
2nd. week of May	6.5	6.5	6.8	6.7	6.62
4th week of May	6.5	6.7	6.5	6.5	6.55
2nd week of June	6.5	6.7	6.8	6.5	6.62
4th week of June	6.8	6.5	6.5	6.5	6.57
2nd week of July	6.7	6.5	6.6	6.5	6.57
4th week of July	6.5	6.8	6.8	6.5	6.65
Mean	6.58	6.62	6.67	6.53	-

3. Semen color: The color of bull semen normally were creamy white in 15 samples out of 24 (62.50%) while the color of 9 semen samples (37.50%) were milky white. The results showed that irrespective of season of collection and age of bull, creamy white and milky white were the dominating color of semen collected from Holstein Friesian bulls (Table-3).

Table-3. Color of Holstein Friesian bull semen.

Month of semen collection	Bull-1	Bull-2	Bull-3	Bull-4
2nd. week of May	Creamy white	Creamy white	Milky white	Creamy white
4th week of May	Creamy white	Milky white	Milky white	Creamy white
2nd week of June	Milky white	Milky white	Milky white	Creamy white
4th week of June	Milky white	Creamy white	Creamy white	Creamy white
2nd week of July	Creamy white	Milky white	Creamy white	Creamy white
4th week of July	Milky white	Creamy white	Creamy white	Creamy white

4. Mass Activity: The mass activity of semen collected from May to July was in range of 3 score, with vigorous movement and moderate rapid waves and eddies to 4.00. Mean mass activity was found higher (4.00), when semen collected in May and June. However; the mass activity of semen was lower as 3.25, when collected in month of July. However, the difference in mass activity either between bulls or between ejaculates months were non-significant (P>0.05) (Table-4).

Table-4. Mass activity of Holstein Friesian bull semen.

Month of semen collection	Bull-1	Bull-2	Bull-3	Bull-4	Mean
2nd. week of May	++++	++++	+++	++++	3.75
4th week of May	++++	++++	++++	++++	4.00
2nd week of June	+++	++++	++++	++++	3.75
4th week of June	+++	++++	++++	++++	3.75
2nd week of July	++++	++++	+++	++++	3.75
4th week of July	+++	++++	+++	+++	3.25
Mean	3.5	4.00	3.50	3.83	-

Microscopic characteristics of fresh and post-thaw semen of Holstein Friesian bull:

Fresh and post-thaw sperm motility:

The average sperm motility from the pooled semen samples in fresh semen was 74.58% against post-thaw sperm motility of 51.87% of Holstein Friesian bull semen. The sperm motility of fresh vs post-thaw semen was obtained as 72.80 vs 48.75%, 75.00 vs 48.75%, 76.25 vs 55.00%, 73.75 vs 52.50%, 75.00 vs 56.25% and 75.00 vs 50.00% during the month of May, June and July respectively. Analysis of variance showed non-

significant effect of season on sperm motility. Fresh and post-thaw semen showed significant difference on sperm motility. The higher sperm motility was recoded as 76.25% in fresh semen and lower 55.00% in thawed semen of Holstein Friesian bull semen in July. In case of post-thaw semen, higher sperm motility of 56.25% was recorded in semen ejaculated in July, while lower 48.75% in May (Table-5).

Table-5: Sperm motility (%) of fresh and post-thaw Holstein Friesian bull semen

Month of semen collection	Mean sperm motility of fresh semen (%)	Mean sperm motility of post-thaw semen (%)
2nd. week of May	72.80	48.75
4th week of May	75.00	48.75
2nd week of June	76.25	55.00
4th week of June	73.75	52.50
2nd week of July	75.00	56.25
4th week of July	75.00	50.00
Mean	74.58	51.87

Sperm concentration:

The higher sperm concentration was recorded as $1549.75x10^6$ in semen ejaculated in month of June, followed by May ($1406.25x10^6$) and July ($1259.50x10^6$) in Holstein Friesian bull semen (Table-6). A significant difference in sperm concentration was found between the ejaculate having. The sperm concentration varied from bull to bull and within bulls and between months of ejaculations collected.

Table-6: Sperm concentration of Holstein Friesian bull semen.

Month of semen collection	Bull-1	Bull-2	Bull-3	Bull-4	Mean
2nd. week of May	$1290x10^6$	$1231x10^6$	$1302x10^6$	$1345x10^6$	$1292.00 x10^6$
4th week of May	$1455x10^6$	$1456x10^6$	$1360x10^6$	$1354x10^6$	$1406.25 x10^6$
2nd week of June	$1491x10^6$	$1542x10^6$	$1432x10^6$	$1415x10^6$	$1470.00 x10^6$
4th week of June	$1629x10^6$	$1503x10^6$	$1552x10^6$	$1515x10^6$	$1548.75 x10^6$
2nd week of July	$1444x10^6$	$1543x10^6$	$1460x10^6$	$1430x10^6$	$1469.25 x10^6$
4th week of July	$1218x10^6$	$1220x10^6$	$1250x10^6$	$1350x10^6$	$1259.50 x10^6$
Mean	$1421.16x 10^6$	$1415.83x 10^6$	$1392.66x 10^6$	$1401.50x 10^6$	-

Membrane Integrity:

The mean membrane integrity of the pooled semen of fresh semen over a period of three months was 58.37% against post-thaw membrane integrity of 44.79%. That membrane integrity in fresh semen was recorded as 60, 75, 57, 58.75, 57.50, 56.25 and 60% ejaculated in May, June and July respectively, while membrane integrity in post-thaw semen in month of May, June and July was 46.25 and 45.00, 43.75 and 42.50, 45.00 and 46.25% respectively (Table-7). It was found that the post-thaw membrane integrity of semen decreased considerably compared to fresh semen.

Table-7: Membrane integrity test of fresh and post-thaw Holstein Friesian bull semen (HOT).

Month of semen collection	Membrane integrity of fresh semen (%)	Membrane integrity of post-thaw semen (%)
2nd. week of May	60.75	46.25
4th week of May	57.00	45.00
2nd week of June	58.75	43.75
4th week of June	57.50	42.50
2nd week of July	56.25	45.00
4th week of July	60.00	46.25
Mean	58.37	44.79

Discussion:

Fresh and post thaw quality of Holstein Friesian bull semen was evaluated for ejaculate volume, pH, mass activity, sperm concentration sperm motility and membrane integrity. Before discussing the results, few major points must be noted. 1. The study was carried out in summer over a limited period i.e. May to July. 2. The member of available bulls was less i.e. 04. 3. There was a little variation recorded in temperature and humidity during the study period.

Semen volume:

The findings of the present study showed that the ejaculate semen volume of Holstein Friesian bulls was recorded within the range of 6-8 ml and differences in ejaculates were significant ($P<0.01$). The volume was significantly ($P<0.05$) higher (7.75 ml) when semen collected in July and slightly decreased (7.25 ml) in May. The results of current study were in agreement to the results reported by Hossain *et al.* (2012). They reported the average volume as 9.3ml in local breeds and 7.4 ml in crosses of Shahiwal×Friesian. But the volume recorded by same author in same study were higher (9.8 -12.8 ml) in Holstein Friesian and crosses of Friesian than the results recorded in current study. These results of present study were partially also fall in the same trend as reported by Koonjaenak *et al.* (2007) and Shaha and Singh (2002). They reported that the ejaculate volume 4.1-7.6 ml for Holstein Friesian cross and Zebu cattle. These results reported by Andrabi *et al.* (2008) for the volume of bull semen averaged 4.92±0.23 ml per ejaculate, were lower than the results of current study.

In fact, volume of semen varies from breed to breed and influenced by a number of factors such as age, breed, weight and season (Ahmed *et al.* 1993; Raja and Rao 1982). Laing *et al.* (1988) reported that a bull of high fertility produced greater semen volume than that in a lower fertility bull. Thus, volume of an ejaculate may be a good indicator of fertility. The Holstein Friesian bulls produced lower ejaculatory volume during dry summer season, whereas higher ($P<0.05$) volume during wet summer compared to other seasons. In another study Fuerst *et al.* (2006) found that the bull breed and environmental temperature had significant effect of on the ejaculated volume of semen. The ejaculate volume was significantly higher in Holstein Friesian and Jersey bulls during stress free and wet summer. Javed *et al.* (2000) noted lowest ejaculate volume in humid summer. However, Fonseca (1995) and Mathevon *et al.* (1998) reported that season had no any significant effect on ejaculate volume for Nelore and Holstein bulls respectively. The differences in findings might be attributed due to differences in breed of bulls and environmental conditions. The bulls of the current study were very young. The discrepancy might be due to the breed variation or age of the bulls.

Color:

In the present study, most of the semen samples were creamy white and milky white in color. These results were in line with those of Koonjaenak *et al.* (2006), they reported that the color of semen of cow bulls was creamy white to milky white, but influenced by ambient temperature and humidity. Kanchan and Matharoo (2015) reported that in majority, of the color of the ejaculates were light creamy to dense creamy. Bhoite *et al.* (2008) also indicated that in all crossbred bulls, creamy color of semen was dominant followed by white and yellow; their findings were almost in similar trend to observation of the present study. The difference in colors of semen ejaculates also pertain to the type of the breed of animal, and management and feed were fed to the animals.

Semen pH:

The pH of semen was recorded as 6.65, it was higher when semen collected in the month of July and slightly lower (6.55) in May ejaculates. The results showed that the semen of Holstein Friesian bulls did not have considerable variation in pH from May to July. The results of current study were in agreements with the results reported by Khan *et al.* (2017) and Anderson (2004). They reported that the mean pH of semen was 6.73±0.02. Hossain *et al.* (2012) reported the average pH before freezing was similar ($P>0.05$) for all breeds and the mean value was 6.4 to 6.5. The results of current study fall in the same trend as observed by Shaha *et al.* (2008), he found that the pH of Holstein Friesian and cross of Zebu cattle semen varied from 6.1-6.5. Therefore, the result of the current study and other relevant findings indicate that pH of semen is not markedly influenced by the variation due to breed.

Normal pH of bull semen is 6.7, when semen stored at room temperature may decreased the pH due to production of lactic acid from fructose. Semen pH may become alkaline when dead sperms percentage were high (Qureshi, 2011). The maximum average pH was obtained from cross-bred bull semen after thawing. The results of present study were also supported by the results of Hossain *et al.* (2012) for cross-bred bull semen. The discrepancy might be due to breed variation and management patterns. However variation in pH values found in the current study might be due to the actively of sperm producing more lactic acid making semen acidic, but it was not within the lethal level.

Mass activity:

The mass activity score of semen samples ejaculated showed vigorous movement with moderate to rapid waves and eddies. Motility is one of the most important requirements of fertile semen. The mass motility of semen was observed significantly lower ($P<0.05$) during wet summer (3.26) than dry summer (4.00). Individual motility in the semen of Holstein Friesian bulls did not differ among seasons ($P>0.05$), but it was lower during wet summer compared to other seasons. The sperm motility of bull spermatozoa recorded in this study was in the similar range to the results reported earlier (Sarder, 2007). The results of the present study were in agreements with the findings of previous studies they found significant changes in progressive motility of sperm at different semen collection times (Fiaz *et al.*, 2010; Sarder, 2007). Seasonal pattern of mass motility and individual motility of semen was different between the bulls and season of semen collection. The results of the present study were comparable with in the range of the above researchers. The lower motility percentage was less than half as effective in producing optimum conception rate. Sarder, (2007) reported the motility of spermatozoa is one of the best single evidence of viability. Fiaz *et al.* (2010) found no significant difference in fertility of semen containing 55 to 95% live sperms, however; semen containing less than 20% of live sperms were considered as infertile. The results of this study indicated that wet summer season deteriorated semen quality in terms of mass motility in the semen of Friesian bulls.

Sperm concentration:

The ejaculate quality of semen was estimated for sperm concentration. The higher sperm concentration of $1548.75x10^6$ was recorded in semen ejaculated in month of June and lower concentration ($1259.50x10^6$) in July. The sperm concentration is generally not associated with the season of semen collection, but it is generally associated with the breed, weight and the age of the bull (Das and Sarkar, 2006). The sperms number per ejaculate differs significantly from bull to bull and within the bulls (Das and Sarkar, 2006) and it ranged between 1 to $4X10^9$ sperm cells/ml. The normal concentration of spermatozoa could be in the range of 150-200 $\times$ 10^6/ ml. These findings were in line with the results recorded in current study in Holstein Friesian bull semen. The results of current study were in close agreements to the results reported by Tomar and Gupta (1984). They reported that the initial motility and sperm concentration were significantly ($P<0.05$) higher during summer season than cold season.

The initial motility and sperm concentration was higher in summer (1193±52.2 million/ml) and lower in winter season (822.7±39.9 million/ml) (Hossan *et al.*, 2012). These results supports to the findings of the present study. In contrast to the results recorded in current study; the Kibria *et al*. (1997) reported highest sperm concentration in spring and lowest in summer; the season had significant ($P<0.05$) effect on sperms concentration and motility. Amir *et al.* (1982) reported that the bull and season had significant effect on the semen volume, sperm concentration and sperm motility before and after freezing. However, season had no significant effect on sperm motility as reported by Hussain *et al.* (1985) and Sarder *et al.* (2000). Seasonal effects may cause by several factors such as ambient temperature, relative humidity, day length and food quality (Mathevon *et al.*, 1998). Sperm concentration in ejaculate is one of the important criteria of semen characteristics to qualify fertile males for breeding purposes (Graffer *et al.*, 1988). Sperm concentration in semen could be considered as an initial indicator of semen quality in semen used for cryopreservation (Shelke and Dhami, 2001).

Post thawing assessment of semen:

Motility and plasma membrane integrity are important aspects to assess the fertility of sperms

after freezing and thawing. In the current study the post thaw motility and integrity of plasma membrane was studied to assess the post thaw fertilizing ability of sperms. Several parameters were used to evaluate the physiological state of sperm after freezing and thawing particularly the motility and membrane integrity. Freezing and thawing of semen leads to the decrease in the percentage of intact sperms and reduces 50% viable sperms.

Sperm motility of fresh and post thaw semen:

The sperm motility was observed as markedly reduced after freezing and thawing as compared to sperm motility of the fresh semen. The higher motility percentage was observed during the month of June, while lower during May in fresh and thawed semen. The average sperm motility of pooled semen samples over a period of three months in fresh semen was 74.58% against post-thaw sperm motility of 51.87%. The sperm motility of fresh vs post-thaw semen was studied and the results were obtained as 72.80 vs 48.75% and 75.00 vs 48.75% in May, 76.25 vs 55.00% and 73.75 vs 52.50% in June and 75.00 vs 56.25 and 75.00 vs 50.00% in month of July. In case of post-thaw semen, higher sperm motility of 56.25% was recorded in semen ejaculated in July, while lower 48.75%, when semen ejaculated in May. The result of the current study were in close agreements to the results reported by Zahid *et al.* (2007). Freezing and thawing of semen leads to the decrease in the percentage of intact sperms and reduces 50% viable sperms. These results were in agreements to the results reported by Siddique *et al.* (2006). Whereas; Holt (2000) reported the motility of semen averaged 45.9 ± 5.2%, which was lower than the findings recorded in current study in Holstein Friesian bull semen. The sperms motility can be associated with better nutrition of bulls, breed as well as environmental temperature of the particular areas where the study conducted.

Membrane integrity test:

The membrane integrity of pooled fresh semen samples over a period of three months was recorded as 58.37% against post-thaw membrane integrity of 44.79%. The membrane integrity has positive correlation with fertility rate in cattle. The integrity of plasma membrane is one of the most important parameter, which is considered to be the key role in success of fertilization, when frozen semen is used (Leite *et al.*, 2010; Lessard *et al.*, 2008). They reported that fertility of frozen-thawed bull semen was reduced by cryopreservation. Andrabi *et al.*, (2009) examined the semen of Sahiwal cow bull and they reported there was no significant difference in post-thaw motility of semen. Furthermore; they reported the fertility rates of frozen semen of Sahiwal bull was 78.78%. This indicates that semen from Holstein Friesian, freezes in similar pattern to those of other breeds of cattle in hot season. However, some contradictory reports were noted from different parts of the world. Such variation could be associated with the feeding regimes, bull breed, management and other environmental factors.

Conclusions:

The ejaculate semen of Holstein Friesian bulls was relatively higher volume. Semen pH did not significantly differ between bulls and ejaculates. Most of the semen samples were creamy white and milky white in color. Mass activity score of semen samples indicated vigorous movement with moderate rapid waves and eddies. Sperm motility was significantly affected in frozen and post-thaw semen.

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