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Evaluation of fresh and post thaw quality of Holstein Friesian bull semen

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Abstract

The study was carried out during the yearr 2017 to determine the fresh and post-thaw quality of four Holstein Friesian bulls. Semen samples were examined for ejaculate volume, pH, mass activity, sperm concentration and sperm motility and sperm membrane integrity (HOST). The ejaculated semen volume of Holstein Friesian bulls was in the range of 6-8ml and differences in ejaculates were significant (P<0.01). The volume was significantly (P<0.05) higher (7.75ml) when collected on 5th July, slightly decrease in volume (7.25ml) when collected on 12th May and 26th July. Semen pH was higher (6.65) for 26th July ejaculation and lowest (6.55ml) for 24th May ejaculation. The results indicated that the semen of Holstein Friesian bulls did not have considerable variation in pH during May – July. Most of the semen samples were creamy white and yellow in colour, while few samples were milky white and watery. Mass activity score of semen samples indicated vigorous movement with moderate rapid waves and eddies. The sperm motility of fresh vs post-thaw semen was obtained 72.80 vs 48.75 percent (12th May), 75.00 vs 48.75 percent (24th May), 76.25 vs 55.00 percent (2nd June), 73.75 vs 52.50 percent (21st June), 75.00 vs 56.25 (5th July) and 75.00 vs 50.00 percent (26th July). In case of post-thaw semen, highest sperm motility of 56.25 percent was recorded in semen ejaculated on 5th July, while lowest post-thaw sperm motility of 48.75 percent was observed in semen ejaculated on 12th May and 24th May. Highest sperm concentration of $1549.75x10^6$ was determined in semen ejaculated on 21.06.2017 and lowest concentration ($1259.50x10^6$) in 26.07.2017 collected samples. It was concluded that sperm motility was significantly (P<0.01) affected by semen types (fresh and post-thaw), while sperm concentration was also significantly (P<0.01) affected by ejaculation date and bulls. The membrane integrity (HOST) of the pooled data on fresh semen samples over a period of three months was 58.37 percent against post-thaw membrane integrity of 44.79 percent.

Keywords: *Semen, Post-thaw Quality, Volume, pH, Mass Activity, Sperm Motility, Membrane Integrity*

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INTRODUCTION

Cryopreservation of mammalian spermatozoa has been a challenge in many species of animals. The direct utilization of a cryopreservation media tested for one species to another to produce the same result has been difficult due to differences in the physiological characteristics of semen produced. This is particularly more important in bull semen cryopreservation due to an enzyme called egg-yolk coagulating enzyme (EYCE) found in the seminal plasma that interacts with egg-yolk of the cryopreservation media, causing coagulation and production of toxic substances deleterious to spermatozoa. The mechanism by which the enzyme causes damage to the spermatozoa is attributed to the production of lysolecithin by hydrolyzing lecithin in the egg-yolk Attempts have been made by several workers to minimize the harmful interaction between EYCE and egg yolk either by removal of seminal plasma through washing (1, 2) or by adding lower concentration of egg-yolk into the extender (3, 4, 5) all with variable outcomes. Despite the possible difference in the type of egg-yolk used, most past research appeared to overlook its importance and impact on the outcome of cryopreservation.

Research has continued, yet with no standard cryopreservation media for goat semen. The type of egg-yolk used and concentration added to extenders remains a great concern in the success of goat semen cryopreservation. Growing interest in improving the quality of frozen bull semen for the needs of the market in recent years has been determined by the declined fertility of dairy cows (4). and world-wide practical usage of artificial insemination (6). Therefore, it is necessary to ensure optimal genetic sires selection (7) as well as semen processing including semen collection (8), dilution (9), slow cooling to 4–6°C. and equilibration (10) as well as cryopreservation maintaining spermatozoa fertilization capability (11) evaluated objectively if possible (12).

The success of cryopreservation depends on the course of cooling and freezing (13). Interaction of temperature vs extender used affects spermatozoa cold shock resistance (14). The extent of spermatozoa damage caused by cold shock depends on temperature, however on the rate of temperature decrease as well. Generally, the higher the cooling rate, the more serious the sperm damage (15) determining the subsequent post-thaw spermatozoa motility (16). Thus the result of sperm cryopreservation is also affected by the type and course of freezing curve (16). Summarily, the length of equilibration as well as the freezing curve type are responsible for many important physicochemical changes leading to different degrees of spermatozoa structure damage (9) deteriorating spermatozoa characteristics after thawing (16). Despite this fact, interactions mentioned have not been sufficiently known yet.

Bull fertility depends partially on the quality of the spermatozoa (i.e., different spermatozoa defect) produced in the ejaculate, emphasizing the importance of the interpretation of the spermatozoa of the result. Production of normal and abnormal spermatozoa and the influence that a spermatozoon defects may have adverse effects on bull fertility (17). Bull sperm were showing to aerobic condition during processing before freezing,and they have tiny endogenous antioxidant to defend them against reactive oxygen classes that may be existing. Seventeen Lab. studies and two pitch trials were conducted with 174 semen collections from bulls in an artificial breeding cooperative. Optimal arrangements consuming an egg yolk-based semen extenders. Amikacin can conflict organisms. Variance extender effect on post thaw semen quality then indicate that altering extender structure or sequence of addling extender component may recover post thaw quality of cryo preserved sperm (16). It would seem that program towards check sperm in total milk extenders the equal day of collection or the day next semen collection should produce equivalent results..A rudimentary tail stump, electron microscopy of ejaculated sperm and testicular tissue from one of the bulls has exposed that a dysfunction of the controller machine throughoutSpermatogenesis stays probable to be the origin of the failing. In expostulated malfunctioning sperm the centriolar adjunct. In the flawed spermids the distal centriole has absent the faculty to form axonemal fibers, so that no end realization can take dwelling.

METHODOLOGY

Selection of Bulls

Total four bulls of Holsten Freezen breed were selected for semen collection.

Semen Collection

Representative bulls of Holsten Freezen were the study models, and semen was collected by at lease 4 different bulls and from each bulls, two ejaculates per bull per week by adopting standardized practices and feed chart for the semen was collected before sunrise. 24 ejaculates of at least 4 different bulls from Holsten freezen breed were collected

The sperm motility of fresh vs post-thaw semen was obtained 72.80 vs 48.75 percent (12.05.2017), 75.00 vs 48.75 percent (24.05.2017), 76.25 vs 55.00 percent (02.06.2017), 73.75 vs 52.50 percent (21.06.2017), 75.00 vs 56.25 (05.07.2017) and 75.00 vs 50.00 percent (26.07.2017).

Dilution Extenders

Different biological extenders were used and their effect on the motility of sperm.

1. Tris + citric acid + Fructose + egg yolk Extender.
2. Sodium citric + egg yolk Extenders
3. Lactose + Fructose + egg yolk Extender
4. Tris + citric acid + Fructose + lactose + egg yolk Extender.

Use of modern biological technique applications for designed study was utilized.

Data Analysis

The collected data was analyzed by applying SPSS statistical software.

RESULTS

Semen volume (ml)

Fresh semen of Holstein Friesian bulls was collected through artificial vagina technique (two times a month) for a three months period (May-July) and ejaculate volume was measured (Table I). The average ejaculate volume of semen of four bulls was significantly ($P<0.05$) higher (7.75ml) when collected on 5th July, while observed slight decrease in ejaculate volume (7.25 ml) equally when collected on 12th May or 26th July. The ejaculate semen volume found a marked decrease i.e. 7.00, 6.75 and 6.62ml averagely for four bulls when semen was collected on 2nd June, 21st June and 24th May. The differences in ejaculate semen volume when collected on 2nd June, 21st June and 24th May were non-significant ($P>0.05$); while ejaculate semen volume did also not differ significantly ($P>0.05$) when collected on 12th May or 26th July. The average ejaculate semen volume of Bull 1, 2, 3 and 4 for six observations was 7.00, 7.00, 7.33 and 7.08ml, respectively showing non-significant ($P>0.05$) variation among the bulls. This indicates that semen volume can vary when expostulated at different time intervals.

Table I. Semen volume per ejaculate (ml) of four Holstein Friesian bulls collected on six dates over three months period (May-July).

Date of semen collection	Bull-1 (Matrix)	Bull-2 (Asian wish)	Bull-3 (Shahzor)	Bull-4 (Khojak)	Mean
12th May 2017	7.00	7.50	7.50	7.00	7.25 ab
24th May 2017	6.00	6.50	7.00	7.00	6.62 b
2nd June 2017	7.50	7.00	7.00	6.50	7.00 b
21st June 2017	6.50	6.50	7.00	7.00	6.75 b
5th July 2017	8.00	7.50	6.00	7.50	7.75 a
26th July 2017	7.00	7.00	7.50	7.50	7.25 ab
Mean	7.00	7.00	7.33	7.08	-

Semen pH

Fresh samples Holstein Friesian bull semen were examined for pH and the data icollected is shown in Table II. The mean pH of the semen samples collected from four bulls was relatively higher (6.65) when collected on 26th July, while a slight variation in semen pH was observed when samples were collected on 12th May and 2nd July with semen pH of 6.62 and

2.62, respectively. Semen pH was equal (6.57) when the bulls were ejaculated on 21st June and 5th July, respectively; and comparatively semen of lowest pH (6.55) was observed when ejaculated on 24th May. The mean semen pH ejaculated from Bulls 1, 2, 3 and 4 for six observations was 6.58, 6.62, 6.67 and 6.53, respectively indicates non-significant (P>0.05) variation among the bulls. Similarly, the variation in pH of semen ejaculated at different dates of three months period was also non-significant (P>0.05). The results indicate that the semen of Holstein Friesian bulls did not have considerable variation in pH during May – July.

Table II. Semen pH of four Holstein Friesian bulls collected on six dates over three months period (May-July).

Date of semen collection	Bull-1 (Matrix)	Bull-2 (Asian wish)	Bull-3 (Shahzor)	Bull-4 (Khojak)	Mean
12th May 2017	6.50	6.50	6.80	6.70	6.62
24th May 2017	6.50	6.70	6.50	6.50	6.55
2nd June 2017	6.50	6.70	6.80	6.50	6.62
21st June 2017	6.80	6.50	6.50	6.50	6.57
5th July 2017	6.70	6.50	6.60	6.50	6.57
26th July 2017	6.50	6.80	6.80	6.50	6.65
Mean	6.58	6.62	6.67	6.53	-

Semen colour

The colour of bull semen is normally determined by visual examination of an experienced person and semen sampled obtained from Holstein Friesian bulls (Table III) indicated that the colour of semen in 12 samples out of 24 (50%) was creamy white, while the colour of 9 semen samples out of 24 (37.50%) was yellow and two semen samples out of 24 (8.33%) had milky white colour. The colour of one sample out of 24 (4.16%) was watery. The results showed that irrespective of age of bull, creamy white and yellow were the dominating/common colour of semen collected from Holstein Friesian bulls.

Table III. Colour of semen ejaculated from four Holstein Friesian bulls on six dates over three months period (May-July)

Date of semen collection	Bull-1 (Matrix)	Bull-2 (Asian wish)	Bull-3 (Shahzor)	Bull-4 (Khojak)
12th May 2017	Creamy white	Creamy white	Yellow	Watery
24th May 2017	Creamy white	Yellow	Yellow	Creamy white
2nd June 2017	Milky white	Yellow	Yellow	Yellow
21st June 2017	Yellow	Creamy white	Creamy white	Creamy white
5th July 2017	Creamy white	Yellow	Creamy white	Creamy white
26th July 2017	Milky white	Creamy white	Creamy white	Yellow

Mass Activity

The mass activity was evaluated in a drop of fresh undiluted semen placed on a pre-warmed slide without cover-slip at low magnification (100×) and scored into 1- 4 scales. The results in Table IV showed that the mass activity of semen collected from four Holstein Friesian bulls at six different dates during a period of three months i.e. May-July was in the range of 3 indicates vigorous movement with moderate rapid waves and eddies. Mean mass activity (on the basis of 1-4 score) was highest (4.00) in semen collected on 24th May and 2nd June 2017 indicating the dense, very rapidly moving waves and eddies, while the mean score of mass activity for semen samples collected on 12th May, 21st June and 5th July was equally 3.75 also reflect dense, very rapidly moving waves and eddies. However, the mass activity score of semen samples collected on 26th July 2017 was 3.25 indicating vigorous movement with moderate rapid waves and eddies. However, statistically, the differences in mass activity either between bulls or between ejaculation dates were non-significant (P>0.05).

Table IV. Mass activity (+ to ++++) of semen ejaculated from four Holstein Friesian bulls on six dates over three months period (May-July).

Date of semen collection	Bull-1 (Matrix)	Bull-2 (Asian wish)	Bull-3 (Shahzor)	Bull-4 (Khojak)	Mean
12th May 2017	++++	++++	+++	++++	3.75
24th May 2017	++++	++++	++++	++++	4.00
2nd June 2017	++++	++++	++++	++++	4.00
21st June 2017	+++	++++	++++	++++	3.75
5th July 2017	++++	++++	+++	++++	3.75
26th July 2017	+++	++++	+++	+++	3.25
Mean	3.66	4.00	3.50	3.83	-

Fresh and post-thaw sperm motility (%)

The sperm motility before freezing (fresh semen) and after thawing was determined and compared. The motility of sperm was evaluated by placing small drop of diluted semen on a clean, pre-warmed slide, covered with a cover slip and examined at a magnification of 400× under light microscope. Sperm motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement while those showing circling movements or oscillating at one place were regarded as immotile. Two examiners separately followed blind techniques for the evaluation. The average scores of sperm motility given by the two examiners were recorded. Analysis of variance showed that individual bull semen had non-significant effect on sperm motility. Semen types (fresh and post-thaw), had significant effect on sperm motility, highest sperm motility of fresh semen (76.25%) was recorded in semen ejaculated on 02.06.2017, while lowest fresh sperm motility (72.80%) was observed in semen ejaculated on 12.05.2017. In case of post-thaw semen, highest sperm motility of 56.25 percent was recorded in semen ejaculated on 05.07.2017, while lowest post-thaw sperm motility of 48.75 percent was observed in semen ejaculated on 12.05.2017 and 24.05.2017. It was observed that sperm motility was markedly reduced after freezing and thawing the semen when compared with the sperm motility of the same fresh semen samples. The average sperm motility from the pooled data over a period of three months in fresh semen samples was 74.58 percent against post-thaw sperm motility of 51.87 percent.

Table V. Comparative sperm motility of fresh and post-thaw semen ejaculated from four Holstein Friesian bulls on six dates over three months period (May-July).

Date of semen collection	Mean sperm motility of fresh semen (%)	Mean sperm motility of post-thaw semen

		(%)
12th May 2017	72.80	48.75
24th May 2017	75.00	48.75
02nd June 2017	76.25	55.00
21st June 2017	73.75	52.50
05th July 2017	75.00	56.25
26th July 2017	75.00	50.00
Mean	74.58	51.87

Sperm concentration

The ejaculate quality is estimated by the sperm concentration values and sperm number per ejaculate may differ within bulls and between dates of ejaculation. Moreover, sperm concentration in the ejaculate serves as one of the criteria of the semen characteristics to qualify fertile males for breeding purposes. The number of viable bovine spermatozoa deposited in the female reproductive tract has been shown to influence fertilizing ability up to an upper threshold level. Significant differences in sperm concentration at semen collection dates have been recorded and highest sperm concentration of $1549.75x10^6$ was determined in semen ejaculated on 21.06.2017, followed by the sperm concentration in semen samples ejaculated on 02.06.2017 and 05.07.2017 with mean sperm concentration of $1470.00x10^6$ and $1469.75x10^6$, respectively. The sperm concentration was determined relatively lower i.e. $1406.25x10^6$ and $1292x10^6$ when the semen was ejaculated on 24.05.2017 and 12.05.2017, respectively. However, the sperm was determined of lowest concentration i.e. $1259.50x10^6$ and the semen samples were ejaculated on 26.07.2017. The study detected a probable decrease in reproductive performance of cryopreserved semen collected at different dates from May to July 2017.

Table VI. Sperm concentration of semen ($x10^6$/ml) ejaculated from four Holstein Friesian bulls on six dates over three months period (May-July).

Date of semen collection	Bull-1 (Matrix)	Bull-2 (Asian wish)	Bull-3 (Shahzor)	Bull-4 (Khojak)	Mean
12th May 2017	$1290x10^6$	$1231x10^6$	$1302x10^6$	$1345x10^6$	$1292.00x10^6$
24th May 2017	$1455x10^6$	$1456x10^6$	$1360x10^6$	$1354x10^6$	$1406.25x10^6$
2nd June 2017	$1491x10^6$	$1542x10^6$	$1432x10^6$	$1415x10^6$	$1470.00x10^6$
21st June 2017	$1629x10^6$	$1503x10^6$	$1552x10^6$	$1515x10^6$	$1548.75x10^6$
5th July 2017	$1444x10^6$	$1543x10^6$	$1460x10^6$	$1430x10^6$	$1469.25x10^6$
26th July 2017	$1218x10^6$	$1220x10^6$	$1250x10^6$	$1350x10^6$	$1259.50x10^6$
Mean	$1421x10^6$	$1415x10^6$	$1392.66x10^6$	$1401.50x10^6$	-

Membrane Integrity

The sperm membrane integrity is determined by hypoosmotic swelling test (HOST) which is the most useful to give positive correlation with motility score and fertility rate. Generally, it has been observed that the increase in the percentage of membrane integrity is also associated with the age of the sire. The results of the present study in relation to membrane integrity of freeze-thaw survival of spermatozoa is presented in Table-7 which showed that membrane integrity determined by HOST in fresh semen was 60,75, 57.00, 58.75, 57.50, 56.25 and 60.00 percent ejaculated on 12.05.2017, 24.05.2017, 02.06.2017, 21,06.2017, 05.07.2017 and 26.07.2018, respectively; while in membrane integrity in post-thaw semen ejaculated on 12.05.2017, 24.05.2017, 02.06.2018, 21,06.2018, 05.07.2018 and 26.07.2018 was 46.25, 45.00, 43,75, 42.50, 45.00 and 46.25 percent, respectively. It was determined that the post-thaw membrane integrity of semen decreased considerably over the membrane integrity of fresh semen. The mean membrane integrity of the pooled data on fresh semen samples over a period of three months was 58.37 percent against post-thaw membrane integrity of 44.79 percent.

Table VII. Membrane integrity (HOST) of fresh and post-thaw semen ejaculated from four Holstein Friesian bulls on six dates over three months period (May-July).

Date of semen collection	Membrane integrity of fresh semen (%)	Membrane integrity of post-thaw semen (%)
12th May 2017	60.75	46.25
24th May 2017	57.00	45.00
2nd June 2017	58.75	43.75
21st June 2017	57.50	42.50
5th July 2017	56.25	45.00
26th July 2017	60.00	46.25
Mean	58.37	44.79

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