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SUBPOPULATION STRUCTURE AND CHANGES AFTER CRYOPRESERVATION OF SPERMS FROM HIGH AND LOW FERTILITY WATER BUFFALO

ESTRUCTURA DE LAS SUBPOBLACIONES Y CAMBIOS DESPUÉS DE LA CRIOPRESERVACIÓN ESPERMÁTICA EN BÚFALOS DE AGUA CON ALTA Y BAJA FERTILIDAD

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ABSTRACT

The aim of this study was to identify the sperm subpopulation structure in buffalo bulls with high and low fertility and to determine how sperm subpopulations change after semen cryopreservation. Semen was obtained from four bulls with high fertility (HF) and four bulls with low fertility (LF) and was cryopreserved. A total of 64 ejaculates were assessed for their sperm kinematics using computer assisted sperm analyzer (CASA). Ward's Hierarchical Dendogram and K-Means clustering method were used to identify the subpopulations. In experiment 1, two significantly different ($P \leq 0.05$) sperm subpopulations were observed: Subpopulation 1 (SP1): sperms travel longer distances most rapidly and progressively, and Subpopulation 2 (SP2): sperms travel shorter distances slower but highly progressive. A higher percentage of SP1 was found in HF bulls (47.27); whereas, a higher percentage of SP2 was found in LF bulls (54.89). A low negative relationship ($r = -0.18$) was observed for the fertility level and sperm subpopulation structure. This implies that sperms that travel longer distances most rapidly and progressively (SP1) are most likely associated to high fertility, while sperms that travel shorter distances slower but highly progressive (SP2) are associated with low fertility. In experiment 2, based on the change in SP1 after cryopreservation, significantly higher sperm survival was observed in samples from HF bulls (29.97) as compared to those from LF bulls (31.64). During post thaw, there were more SP1 sperms in samples from HF bulls (27.52) than in those from LF bulls (26.74). Thus, semen containing higher proportion of SP1 sperms are more resistant to cryopreservation and have greater chances of obtaining high fertility. Overall, the identification of sperm heterogeneity in water buffaloes can be associated to sperm survival after cryopreservation and fertility.

Key words: Sperm subpopulation; Sperm cryopreservation; Fertility, Water buffalo; CASA.
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RESUMEN

El propósito de este estudio fue identificar la estructura de las subpoblaciones espermáticas en toros bufalinos con alta y baja fertilidad y determinar los cambios luego de la criopreservación. El semen se obtuvo de cuatro búfalos con alta fertilidad (HF) y cuatro con baja fertilidad (LF) y fue criopreservado. Un total de 64 eyaculados fueron evaluados para parámetros cinéticos usando un analizador espermático asistido por computadora (CASA). El método de dendograma jerárquico de Ward y el método K-means fueron utilizados para identificar las subpoblaciones. En el experimento 1, dos subpoblaciones espermáticas estadísticamente diferentes ($p<0.05$) fueron observadas: Subpoblación 1 (SP1): espermatozoides que viajan largas distancias más rápida y progresivamente, y la Subpoblación 2 (SP2): espermatozoides que viajan distancias cortas de forma lenta pero muy progresivamente. Un mayor porcentaje de SP1 fue encontrado en los búfalos HF (47,27); mientras que un mayor porcentaje de SP2 fue encontrado en los búfalos LF (54.89). Una relación baja pero negativa ($r = -0,18$) fue observada para el nivel de fertilidad y la estructura de la subpoblación espermática. Esto implica que los espermatozoides que viajan largas distancias más rápida y progresivamente (SP1) están más asociados a la alta fertilidad, mientras que los que viajan distancias cortas más lento y con alta progresividad (SP2) están asociados a una baja fertilidad. En el experimento 2, en base a los cambios en SP1 luego de la criopreservación, un mayor porcentaje de espermatozoides permaneció en esta subpoblación en los búfalos HF (27,52) que en los LF (26,74). Por lo tanto, semen con una alta proporción de espermatozoides dentro de SP1 son más resistentes a la criopreservación y tienen mayor probabilidad de obtener una mayor fertilidad. En general, la identificación de la heterogeneidad espermática en búfalos de agua puede ser asociada a la sobrevivencia luego de la criopreservación y a la fertilidad.

Palabras clave: Subpoblación espermática; Criopreservación espermática; Fertilidad; Búfalos de agua; CASA.
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INTRODUCTION

The identification of bulls that produce large populations of fertile sperms is important for the Artificial Insemination (AI) industry (Shojaei et al. 2012). However, genetically superior bulls show varying sperm quality that leads to unpredictable fertility (Ward 1998). Characterization of sperm ejaculate heterogeneity based on its kinematic parameters is gaining interest in the livestock sector to determine male fertility levels and cryosurvival (Ramón et al. 2013; Yániz et al. 2015) Identification of sperm subpopulation is achieved using clustering statistical methods to distinguish sperms sharing common characteristics (Martinez-Pastor et al. 2011). Theoretically, a greater heterogeneity of spermatozoa results in a greater chance for the sperm to fertilize the oocyte (Curry et al. 1994). Results from methods assessing sperm heterogeneity have been shown to be significantly correlated with fertility in boars (Abaigar et al. 1999; Quintero-Moreno et al. 2004), bucks (Dorado et al. 2010), gazelles (Abaigar et al. 1999, 2001), and stallions (Quintero-Moreno et al. 2004), but studies in water buffaloes are still focused on the use of means in identifying the sperm characteristics that provides little information.

Studies using computer assisted sperm analyzer (CASA) to determine the sperm quality focusses on the velocity, travelled distance, head movement and trajectory as traits that influence fertility. This support the importance of a use of multi-parametric test in the identification of bulls with high and low fertility (Ramón et al. 2013). Furthermore, CASA objectively assess sperm motion characteristics that leads to high accuracy and repeatability, which can be more indicative of the quality of the sample (Awad, 2011).

To evaluate fertility, factors like number of females inseminated, number of bulls used for collection, amount of spermatozoa analyzed, and freezing method have to be considered for a more valid estimation (Amann, 1989). Cryopreservation induce the loss of sperm viability and functionality (Watson, 1995) and affect several motility parameters evaluated by CASA, like, path velocity, curvilinear velocity, lateral amplitude of head movement and beat cross frequency (Sundararaman et al. 2012), thus it has also been linked to the failure of pregnancy.
The importance of identifying sperm attributes related to fertility will complement the selection of bulls using the traditional breeding soundness and estimated breeding values evaluation. The significance of this research is that characteristics that account for high fertility will be used in identifying potential bulls for cryopreservation and breeding, while with low fertility bulls, a better strategy may be devised to improve field fertility (Kumar et al. 2014).

Therefore, the present study aims to characterize the sperm subpopulations structure of buffalo bulls (Bubalus bubalis) with high and low fertility, as well as to determine the effect of cryopreservation on the composition of sperm subpopulations in high and low fertility buffalo bulls.

MATERIALS AND METHODS

Chemicals and supplies

All chemicals used in this study were purchased from Sigma Chemical Company (Sigma, St Louis, MO, USA) unless otherwise stated. Fresh egg yolk for the semen extender was obtained from a local poultry farm. Leja slides were obtained from IMV Incorporated (Leja Products B.V., Luzernestraat 10, 2153 GN Niewuw Vennep, The Netherlands).

Selection of animals

Eight Bulgarian Murrah bulls with ages ranging from six to ten years old, maintained in uniform feeding and housing conditions at a Semen Collection and AI Center, were selected for this study. All bulls were being used in breeding based on the selection method of the genetic improvement program of the Center. These bulls were classified as either high fertility (HF) or low fertility (LF), four each, on the basis of the four-year AI pregnancy rate of the Center as seen on Table 1. According to industry standards, bulls with a fertility score that is higher by 3% than the breed average (31.59%) is considered as high fertility bull, whereas bulls with a fertility score lower by 3% than the breed average is denoted as low fertility bull (Shojaei et al. 2012).

Semen collection and cryopreservation

Four successive collections with one-week interval each, were made for all bulls using an artificial vagina and teaser bull. A total of 64 samples, 2 ejaculate from each bull were collected and processed. At the onset, ejaculates were assessed for volume, color, pH, sperm concentration, and subjective motility grading. Aliquot of the fresh sperms were taken and placed on a water bath for the fresh semen CASA evaluation, while the remaining semen was processed for cryopreservation. The standard cryopreservation protocol of the Semen Processing Center was followed using a Tris-egg yolk-fructose-glycerol (TYFG) sperm extender and cryoprotectant. During the equilibration stage, aliquot of the semen

were again taken for the CASA evaluation for the pre-freeze stage. Finally, semen freezing was done using a simple rapid freezer (FHK, FA-1652). Post thaw evaluation using CASA was done 24 hours to one week after freezing.

Table 1. Individual record of pregnancy rate of eight bulls at Philippine Carabao Center at Central Luzon State University (4 years).

Bull	No. of Inseminations	Calves produced	%Production (Pregnancy)	Fertility level
1	2321	865	37.27	High
2	336	159	47.32	High
3	2546	1012	39.75	High
4	4268	1564	36.64	High
5	672	190	28.27	Low
6	666	163	24.47	Low
7	436	113	25.92	Low
8	2512	649	25.84	Low

CASA analysis

The aliquots of semen used for CASA evaluation that were taken during the fresh, pre freeze and post thaw stages were diluted in normal saline (sperm concentration adjusted to 25 million sperms/ml) and held in a dry bath at 38°C for 15-20 mins before evaluation. Samples were analyzed using a Hamilton Thorne IVOS II ver. 14 Computer Assisted Sperm Analyzer (Hamilton Thorne-IVOS, Bedfrod, MA). The analysis set-up used to evaluate the buffalo sperm is presented in Table 2. Using a Leja slide, 3µl of the prepared semen sample were loaded and analyzed on the CASA machine with seven microscopic fields each, recording at least 100 motile sperms.

Table 2. Technical setting of CASA (Hamilton-Thorne IVOS 14) for motility assessment of buffalo sperm.

Parameters	Set value
Frame rate (Hz)	60
Frames acquired	30
Minimum contrast	80
Minimum cell size (pixels)	5
Cell size (pixels)	5
Cell intensity (pixels)	70
Path velocity (VAP) (um/s)	50
Straightness (STR) (%)	70
VAP cut-off (um/s)	25
VSL cut-off (u/s)	15

The semen samples were analyzed through CASA based on the travelled distance, velocity, head movement, and trajectory. The measured sperm kinematics were average distance path (DAP, µm), distance curved line (DCL, µm), distance straight line (DSL, µm), average path velocity (VAP, µm), curvilinear velocity (VCL, µm), straight line velocity (VSL, µm), amplitude of lateral head movement (ALH, µm), beat cross frequency (Hz), linearity (LIN, %), straightness (STR, %) and wobble (WOB, %).

Statistical analysis

The statistical analysis used in the study was based on the research of Rencher (2005) with minor modifications. Dendograms were created using the Ward's Method of Hierarchical Clustering to determine the appropriate number of clusters to be formed with an acceptable distance. Meanwhile, a multivariate k-means cluster analysis was performed for the classification of the sperms into a reduced number of clusters or subpopulations according to their movement. The values for each sperm parameter is presented as mean±SE, whereas population of sperms belonging to each cluster are computed and shown in frequencies. Spearman's correlation was done to establish the relation between the classification of bull according to the level of fertility (high or low) and the classification of sperm samples in Subpopulation 1 or Subpopulation 2. T tests were carried out to compare the two sperm subpopulations based on the individual sperm kinematics. A two-way multi-variate analysis of variance (MANOVA) and Wilk's Lambda test was used to determine the interaction of fertility and cryopreservation. Chi square was used to compare the percent frequency distribution of the subpopulations according to fertility and cryopreservation stages. The statistical level of significance was set at $P \leq 0.05$ using SSPS Statistics Data Editor version 19.0 (SPSS Inc., Chicago, IL, USA).

The two experiments carried out in the study were:
Experiment 1: Motility characteristics of sperm subpopulations of high and low fertility bulls (combined data from fresh and frozen thawed semen); and
Experiment 2: Effect of cryopreservation on composition of sperm subpopulations.

RESULTS

Motility characteristics of sperm subpopulations of water buffalo bulls

There were significant differences ($P \leq 0.05$) on each of the kinematic parameters on the two sperm subpopulations identified in water buffalo bulls as seen on Table 3. Sperm Subpopulation 1 had high values for the distance (DAP, DSL, and DCL), velocity (VAP, VSL, and VCL), and trajectory parameters (STR, LIN, and WOB). It also had high BCF and moderate ALH (head movement). Generally, those sperms travelled longer distances most rapidly and progressively. Sperm Subpopulation 2, on the other hand, had low values for the distance and velocity parameters, high values for trajectory and BCF, and low values for ALH. These sperms travelled slower covering shorter distances but were highly progressive.

Table 3. The difference on the sperm kinematics of each sperm subpopulations according to fertility level.

Kinematic Parameters	Sperm subpopulations					
	High			Low		
	1	2	P value	1	2	P value
DAP (µm)	55.01±0.15^a	23.67±0.09^b	0.000	53.46±0.16^a	25.03±0.10^b	0.000
DSL (µm)	46.25±0.15^a	19.64±0.09^b	0.000	45.12±0.16^a	20.94±0.10^b	0.000
DCL (µm)	99.07±0.29^a	40.62±0.15^b	0.000	92.81±0.28^a	42.09±0.16^b	0.000
VAP (µm/s)	54.16±0.22^a	58.71±0.21^b	0.000	153.24±0.26^a	61.53±0.22^b	0.000
VSL (µm/s)	128.84±0.28^a	48.93±0.21^b	0.000	127.43±0.29^a	51.64±0.22^b	0.000
VCL (µm/s)	276.06±0.44^a	99.79±0.32^b	0.000	266.27±0.50^a	102.51±0.33^b	0.000
ALH (µm)	10.28±0.02^a	4.73±0.02^b	0.000	10.36±0.02^a	4.84±0.02^b	0.000
BCF (Hz)	32.90±0.07^a	32.59±0.07^b	0.002	31.17±0.07^a	32.14±0.08^b	0.044
STR (%)	83.11±0.13^a	80.58±0.14^b	0.000	83.16±0.13^a	80.47±0.15^b	0.000
LIN (%)	48.18±0.11^a	49.48±0.14^b	0.000	50.00±0.12^a	50.40±0.15^b	0.000
WOB (%)	57.00±0.07^a	59.13±0.10^b	0.000	58.99±0.08^a	60.25±0.11^b	0.000

Mean±SE. Means with the same superscripts on each row are not significantly different from each other at 0.05 level of significance.

Meanwhile, Table 4 presents the frequency distribution of sperm according to sperm subpopulations and fertility. Sperm in subpopulation 2 were more frequent in both high and low fertility bulls, but a significantly higher ($P \leq 0.05$) percentage of rapid and progressively moving (Subpopulation 1) sperms were recorded in HF bulls with a percentage of 47.27 as against to 45.51 in LF bulls. Whereas, the percentage of low velocity or poorly motile with high progressiveness (Subpopulation 2) sperm was significantly greater ($P \leq 0.05$) in LF than in HF bulls (54.89 versus 52.73). The proportions were compared using Chi-square test with a value of 25.387 and P value of 0.000. The Spearman's Correlation show a low negative association between fertility level of the bulls and the subpopulation classification ($r = -0.18$), this is that sperms from HF bulls were more frequently classified in Subpopulation 1 which is most rapid and progressively moving, while those from LF bulls were classified into Subpopulation 2 as low velocity or poorly motile with high progressiveness sperm. In summary, sperms that were most rapid and progressive are most likely to be associated high fertility in water buffaloes.

Table 4. The frequency distribution of sperms according to subpopulation in high and low fertility bulls.

	Subpopulations		
Fertility	**1**	**2**	**Total**
Low (%)	17,849 (45.51)[a]	21,370 (54.89)[a]	39,219
High (%)	20,313 (47.27)[b]	22,663 (52.73)[b]	42,976
Total	38,162	44,033	82,195

Values inside parentheses show percentage comparison on both subpopulations according to fertility. Means with the same superscripts on each column are not significantly different from each other at 0.05 level of significance.

Changes on the Sperm Subpopulations in High and Low Fertility Bulls Across Cryopreservation Process

The changes on the frequency of sperm subpopulations in high and low fertility bulls recorded across cryopreservation is shown in Figure 1. There is a significant difference ($P \leq 0.05$) on the two sperm subpopulations for all the cryopreservation stages except for the pre-freeze of high fertility bulls. A decline in the percent frequency of Subpopulation 1 sperms during cryopreservation at both fertility levels was observed with the concurrent increase in Subpopulation 2 sperms.

Shown in Table 5 are the comparisons on the subpopulations according to fertility and cryopreservation stages. There was a similarly high percentage of most rapid and progressive sperm (Subpopulation 1) on HF and LF level fresh semen (58.38 and 57.49, respectively, $P \geq 0.05$). For the pre-freeze stage, a significantly higher 47.81 percent was observed in HF that in LF, 45.30. Finally, at post thaw, a numerically higher percentage of sperms remained in Subpopulation 1 for HF level (27.52) than for LF level (26.74).

However, looking at the percentage of decline in the value of Subpopulation 1 from fresh to post thaw, there was a higher percentage points reduction in LF level (31.64) than that of the HF level (29.97). The present finding indicates a variation on the sperm kinematics from semen of HF and LF bulls during cryopreservation. Finally, more sperms from HF bulls were able to remain within Subpopulation 1 after cryopreservation than sperm from low fertility bulls.

The comparison of means of sperm kinematics for the interaction of fertility and cryopreservation is presented at Table 6. Using Wilk's Lambda MANOVA, a significant interaction was observed with a value of 0.987 and 0.000 P value. These implies that there is a significant interaction between fertility and cryopreservation stages. Hence, there is significant difference on the sperm kinematics between HF and LF with at least one of the cryopreservation stages.

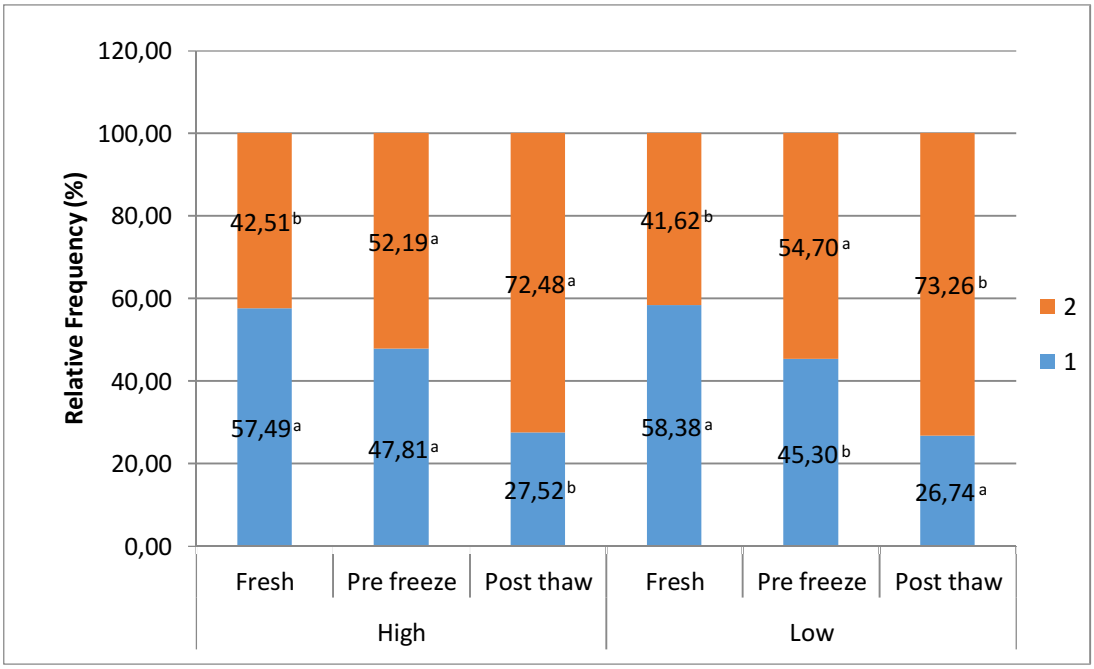

Figure 1. Frequency distribution for each sperm subpopulations from bulls with different fertility levels in the cryopreservation stages. Different letters (a and b) inside the column bars indicate significant differences between subpopulation frequency (P≤0.05).

Table 5. Frequency distribution of sperm subpopulations across cryopreservation stages according to the fertility.

	1		2		P value
	High	**Low**	**High**	**Low**	
Fresh	8,552 (57.49)[a]	8,116 (58.38)[a]	6,324 (42.51)[a]	5,786 (41.62)[a]	0.126
Pre-freeze	9,491 (47.81)[a]	7,231 (45.30)[b]	10,361 (52.19)[a]	8,730 (54.70)[b]	0.000
Postthaw	2,270 (27.52)[a]	2,502 (26.74)[a]	5,978 (72.48)[a]	6,854 (73.26)[a]	0.248

Values inside parentheses show percentage comparison on both subpopulations according to fertility and cryopreservation step. Means with the same superscripts on each column are not significantly different from each other at 0.05 level of significance.

Table 6. Post hoc analysis of the sperm kinematics on the interaction of cryopreservation and fertility level using analysis of variance and Tukey's HSD.

Kinematic Parameters	High			Low		
	Fresh	Pre freeze	Post thaw	Fresh	Pre freeze	Post thaw
DAP (µm)	44.15±0.21^{b}	36.52±0.16^{c}	32.56±0.22^{e}	46.00±0.21^{a}	35.43±0.16^{d}	31.00±0.20^{f}
DSL (µm)	39.33±0.20^{b}	29.06±0.14^{c}	26.61±0.21^{e}	41.39±0.20^{a}	27.75±0.15^{d}	25.50±0.19^{f}
DCL (µm)	74.92±0.37^{a}	67.54±0.31^{b}	57.12±0.40^{d}	75.02±0.34^{a}	63.49±0.31^{c}	54.73±0.37^{e}
VAP (µm/s)	119.11±0.47^{a}	101.47±0.40^{c}	80.61±0.52^{d}	119.28±0.45^{a}	104.95±0.46^{b}	78.73±0.49^{e}
VSL (µm/s)	105.76±0.45^{a}	80.64±0.36^{b}	65.81±0.49^{c}	106.86±0.44^{a}	81.52±0.39^{b}	64.57±0.44^{c}
VCL (µm/s)	201.28±0.83^{a}	186.19±0.77^{c}	140.45±0.93^{d}	194.46±0.76^{b}	187.00±0.90^{c}	138.31±0.86^{d}
ALH (µm)	7.25±0.03^{c}	8.05±0.03^{b}	5.80±0.03^{f}	7.08±0.03^{d}	8.42±0.04^{a}	6.11±0.04^{e}
BCF (Hz)	34.60±0.08^{a}	30.90±0.10^{d}	33.96±0.12^{b}	34.40±0.08^{a}	29.15±0.08^{e}	31.91±0.12^{c}
STR (%)	87.91±0.13^{a}	77.96±0.15^{c}	79.88±0.24^{b}	88.34±0.13^{a}	76.77±0.16^{d}	80.01±0.22^{b}
LIN (%)	54.92±0.14^{b}	44.65±0.12^{e}	48.12±0.22^{c}	56.40±0.14^{a}	45.76±0.21^{d}	48.40±0.15^{c}
WOB (%)	61.25±0.10^{b}	55.69±0.16^{e}	58.37±0.08^{c}	62.68±0.11^{a}	57.69±0.10^{d}	58.47±0.15^{c}

Mean±SE. Means with the same superscripts on each row are not significantly different from each other at 0.05 level of significance

DISCUSSION

Sperm heterogeneity is a widely recognized feature that plays a role in determining the reproductive performance of males. Meanwhile, fertility rate is considered to be the best parameter to assess the quality of semen (Vale, 1997). The intention of this research was to determine the sperm subpopulation in semen of water buffaloes and how these subpopulations change according to the fertility of bulls and after the cryopreservation process.

The results of the study indicate that Subpopulation 1, which has the most rapid and progressive sperms, and sperm in this subpopulation were more frequent in HF than LF (Table 4). This is due to the more sperms found in the HF that move with faster speed, and high straightness and linearity than in LF. This finding is similar to those of Ferraz et al. (2014) and Yániz et al. (2015) claiming that the subpopulation with the most rapid and progressive sperm was the most suitable for being part of the fertilizing population. The ejaculates with higher proportion of rapid and linear moving sperms would probably have more migration that is efficient through the female reproductive tract. In addition, they found that fast and linear spermatozoa with large and long nuclei are related to high fertility. Others have also proposed that sperm belonging to clusters with high velocities (VCL, VAP, VSL) and more progressive movement (high STR and LIN) could be considered as the sperm with the highest fertilizing potential (Cremades, 2005; Núñez-Martínez et al. 2006 ; Quintero-Moreno et al. 2003). This is functionally related to a higher number of sperm bound to the zona pellucida of the oocyte. The distribution of subpopulations between HF and LF rams was significantly different, with a higher proportion of spermatozoa of the SP2mot (rapid and linear) and of the SP3morpho (large and long spermatozoa), and a lower proportion of the SP3mot (rapid nonlinear) in the higher fertility rams than in the lower fertility rams. Rams with superior field fertility displayed increased proportion of spermatozoa with efficient swimming (fast and linear) and with large and long heads (Yániz et al. 2015). According to the result of the present study, in water buffaloes, a majority of rapid and progressively sperm are a good indicator of high fertility bulls.

In terms of the effect of cryopreservation, differences that were observed between good and poor freezer bulls are based solely on the ability of the spermatozoa to withstand drastic changes that happen during the freezing process (Sundararaman and Edwin 2008). In relation to that, in the present study a moderate percentage of sperm changing from Subpopulation 1 to Subpopulation 2 during cryopreservation was observed, with HF bull having a lower reduction of Subpopulation 1 (57.49% to 27.52%) in comparison of LF bulls (58.38% to 26.74%). In bulls, subpopulation of highly active and progressive sperm changed from 34.1% in fresh to 20.6% in thawed semen (Muiño et al. 2008), while in boar, changed from 90.1% to 58.7% (Cremades, 2005). The lower fertility is based on the low membrane phospholipid content of the sperm and its loss during freezing (Galli and Lazzari, 1996). Changes in the proportions of sperm into each subpopulation after

cryopreservation could be relate to the phase transitions and other ultrastructural modifications of the plasma membranes during cooling and rewarming, and this may play a role in the poor fertility of cryopreserved sperm (Bailey et al. 2000), also, redistribution of phospholipids probably affects membrane function and charge, and even lead to severe membrane dysfunction, resulting to lower fertility (Barroso et al, 2000). Meanwhile, according to Shojaei et al. (2012), sperms from HF bulls are in transition to a hyper activated motility pattern, whereas those from LF bulls have only a forward progressive motility pattern, which is similar to the findings of this study. It is also inferred that frozen-thawed sperm from HF bulls were more efficient in undergoing hyperactivation compared to LF bulls. In boars, there is an increase in proportion of hyperactivated sperms during freezing, and these sperms exhibiting progressive and vigorous movement remained constant throughout the cryopreservation procedure which can indicate its resistance. Moreover, cryopreservation resistance of hyperactivated sperm is defined by having a high flagellar bend amplitude and, beat asymmetry at post thaw (Cremades, 2005), which is similar with the characteristic of Subpopulation 1 sperms in HF bulls from this study. Furthermore, Muiño et al. (2008) explain that the ejaculates that are most resistant to cryopreservation and show the best post-thaw sperm longevity contain the highest populations of rapid and progressive sperm. Based on the findings of Freitas et al. (2015) and Muiño et al. (2008), greater values of VAP, VSL, VCL, and ALH obtained at post thaw indicate a hyperactive movement of the spermatozoa giving a higher rate of pregnancy.

CONCLUSIONS

Two sperm subpopulations in HF and LF buffalo bull were identified. SP1 are sperms that travelled longer distances most rapidly and progressively while SP2 sperms travelled slower, shorter distances but were highly progressive. HF bulls were found to be associated with most rapid and progressive sperms while LF to the slower but highly progressive sperms. In addition, cryopreservation was found to affect the percentage of sperm into each subpopulation, decreasing the proportion of most rapid and progressive (SP1) at post-thaw. Finally, HF bulls have HF bulls have sperm with higher capacity to remain into subpopulation 1 after cryopreservation than LF bulls.

The identification of sperm heterogeneity using computer assisted sperm analysis paves the way on establishing sperm subpopulations that are related to fertility and cryopreservation survival. Bulls with semen with higher proportion of rapid and progressive sperm is related to high fertility and can be fully maximized for cryopreservation and breeding purposes for the advancement of genetic improvement in water buffaloes.

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