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Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during low-breeding season

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ABSTRACT

The present study was carried out to assess various postthaw semen quality parameters for the prediction of fertility in buffalo bull during low-breeding season. Semen (30 ejaculates) was collected from five adult buffalo bulls with artificial vagina (42 °C). Sperm motility parameters, velocity distribution, motion kinematics, and subpopulations were analyzed by computer-aided sperm motion analyzer (CASA). Moreover, sperm visual motility, supravital plasma membrane integrity, viability/acrosome integrity, viability/mitochondrial transmembrane potential, DNA fragmentation/integrity, and morphology were analyzed by phase-contrast microscope, supravital hypoosmotic swelling test, Trypan blue/Giemsa staining, propidium iodide/"5,5′,6,6'-tetrachloro-1,1′,3,3'-tetraethylbenzimidazolyl carbocyanine iodide" (JC–1) fluorochromes, neutral comet assay/acridine orange assay and wet mount technique, respectively. Outcome of 528 inseminations was analyzed for *in vivo* fertility. Pearson's correlation coefficients revealed that sperm progressive motility (%), rapid velocity (%), average path velocity (μm/s), straight line velocity (μm/s), subpopulation one (most rapid, and progressive) of motile spermatozoa (%), supravital plasma membrane integrity (%), and viable spermatozoa with intact acrosome (%) were significantly correlated with *in vivo* fertility ($r = 0.64$, $P < 0.01$; $r = 0.57$, $P < 0.01$; $r = 0.52$, $P < 0.01$; $r = 0.56$, $P < 0.01$; $r = 0.73$, $P < 0.001$; $r = 0.74$, $P < 0.001$; $r = 0.88$, $P < 0.001$); whereas nonviable spermatozoa with damaged acrosome or low-mitochondrial transmembrane potential and comet length (μm) of neutral comet assay were negatively associated with *in vivo* fertility ($r = -0.79$, $r = -0.75$, $P < 0.001$, and $r = -0.60$, $P < 0.05$, respectively). Multiple regression analysis reported that combination of semen quality parameters as predictor of fertility were better (R^2 adjusted = 81.30%, $P < 0.001$) as compared with single parameter (R^2 adjusted = 50.20%, $P < 0.007$). It is concluded that assessment of CASA parameters and some other sperm structural and functional parameters, that is, integrity of plasma membrane and acrosome, and transmembrane potential of mitochondria were able to predict the *in vivo* fertility of water buffalo bull during low-breeding season.

1. Introduction

Sperm cryopreservation contributes to the development of reproductive techniques, such as artificial insemination (AI) [1]. Fertility of cryopreserved water buffalo spermatozoa through AI is reported to be affected by seasonality, that is, ambient temperature, relative humidity, and day length in a particular season [2,3]. Several studies [4–9] have reported lower cryodamage and subsequently higher fertility of buffalo spermatozoa during the autumn/winter (peak-breeding season) compared with that processed during the summer (low-breeding season). Postthawing sperm motility (%; 49.64 ± 1.18 vs. 41.78 ± 1.45) was significantly higher in peak than in low-breeding seasons [4]. A similar trend was

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reported by Bahga and Khokar [6] viz. postthawing sperm motility was higher during peak than low-breeding seasons (%; 67.71 ± 2.76 vs. 28.92 ± 2.88, respectively). Likewise at postthaw, plasma membrane integrity (% of alive spermatozoa) was higher in peak (54.6%, $P < 0.001$) than in low (45.1%)-breeding seasons [7]. Regarding the postthaw sperm DNA fragmentation index in swamp buffalo, its values varied significantly among seasons, being lower in humid months (1.40 ± 0.21%) than in the winter (2.16 ± 0.21%) [8]. Similarly in the riverine buffalo, a significant difference in postthaw sperm DNA damage (%; 13.61 ± 1.90 vs. 4.61 ± 1.07) was observed during summer as compared with the winter [10]. A fertility difference of about 10% was observed in cryopreserved buffalo spermatozoa during low- and peak-breeding seasons (32.1 vs. 42.3, respectively, $P < 0.01$) [5]. Later on, Younis et al. [11] also confirmed that the fertility of frozen-thawed buffalo bull spermatozoa was higher during peak than low-breeding seasons (%; 44.87 ± 1.01 and 38.73 ± 1.72, respectively). Hence, this highlights that accurate evaluation of structural cum functional integrity of cryopreserved spermatozoa and to predict its fertilizing capacity is critical for the success of AI [12] in low-breeding season [8].

The various postthaw semen quality tests that can be used for accurate evaluation include (1) computer-aided sperm analysis (CASA) motility parameters, velocity distribution, and motion kinematics, (2) mitochondrial transmembrane potential (% of alive spermatozoa), (3) acrosome integrity (% of alive spermatozoa), (4) DNA damage (by acridine orange and neutral comet assays), and (5) supravital hypoosmotic swelling. However, most of these tests are required to be exploited as fertility predictors of cryopreserved buffalo bull spermatozoa during low-breeding season.

The present study was carried out to assess various postthaw semen quality parameters for the prediction of fertility in buffalo bull during low-breeding season.

2. Materials and methods

2.1. Chemicals

All the chemicals used in this study were purchased from Merck, Darmstadt, Germany.

2.2. Semen collection and initial evaluation

Semen was collected from five mature water buffalo bulls maintained at National Agricultural Research Centre, Islamabad, Pakistan with artificial vagina (42 °C) during low-breeding season (May–July). The month-wise meteorological data of Islamabad (Pakistan) during low-breeding season was: May, ambient temperature (35 °C, 32 °C–38 °C), relative humidity (55.5%, 24%–87%), day length (14.09 hours, 14.21–13.49 hours); June, ambient temperature (39 °C, 37 °C–41 °C), relative humidity (67.5%, 41%–94%), day length (14.33 hours, 14.14–14.21 hours); July, ambient temperature (34 °C, 30 °C–38 °C), relative humidity (75%, 54%–95%), day length (14.19 hours, 14.21–13.49 hours).

After collection, semen samples were transferred to the laboratory immediately for initial evaluation. Sperm visual motility was assessed using phase contrast microscope (Olympus BX20, × 400) connected with closed circuit monitor, and sperm concentration was measured by using the specific spectrophotometer (SDM 5, Minitube, Germany) at 546 nm.

2.3. Semen extension and cryopreservation

Qualifying ejaculates (n = 30) having > 70% visual motility and greater than 0.5×10^9 sperm/mL concentration from each bull were diluted in Tris–citric acid egg yolk glycerol extender at 37 °C, cooled to 4 °C in 2 hours, and equilibrated at 4 °C for 4 hours in cold cabinet unit [13]. Extended semen was then packed in polyvinyl French straws (0.54 mL, IMV, France) and frozen in a programmable cell freezer (Kryo-550, Planer, Middlesex, UK). Finally, semen straws were plunged into liquid nitrogen (−196 °C). The straws were stored in liquid nitrogen tank for at least 24 hours before postthaw analyses. Thawing of semen straws was carried out for 30 seconds at 37 °C.

Two pooled ejaculates of each bull per replicate were used for the postthaw laboratory analyses (five bulls, three replicates, 30 ejaculates, 15 batches). For the fertility trial, cryopreserved semen from the same 15 batches was used.

2.4. Computer-aided sperm motion analysis (CASA)

Frozen-thawed semen samples were maintained at 37 °C for 5 minutes before evaluation. After thorough mixing, semen sample (7 μL) was placed on a prewarmed slide and coverslipped [14,15]. The sample loaded slide was fitted in a portable Mini Therm stage (37 °C) of microscope (× 100) connected to a computer having CASA software (CEROS, version 12.3, Hamilton Thorne Biosciences, USA). Total motility (TM, %), progressive motility (%), rapid velocity (%) medium velocity (%), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR, %; ratio of VSL/VAP) and linearity (LIN, %; ratio of VSL/VCL) were analyzed for at least 200 spermatozoa per sample by using "Standard" settings of CEROS CASA (Table 1).

Table 1
Standard analysis setup of CASA for estimation of sperm quality.

Frames acquired	30
Frame rate	60 Hz
Minimum cell size	5 Pixels
Minimum contrast	56
Motile, VAP cutoff	20 μm/s
Progressive motile	VAP 80 μm/s, STR 80%
Rapid (velocity)	VAP > 80 μm/s
Medium (velocity)	VAP > 20 μm/s < 80 μm/s
Magnification	1.89
Temperature, set	37 °C

Abbreviations: CASA, computer-aided sperm motion analysis; STR, straightnes; VAP, average path velocity.