Assessment of semen quality in Swamp Buffalo AI Bulls in Thailand

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ABSTRACT - Characteristic of Thai swamp buffalo bulls semen used for artificial insemination (AI) in Thailand, aspects relevance in freezing and thawing of semen are review. Semen and sperm characteristics were evaluated included sperm count, motility (assessed subjectively and by CASA), morphology (using phase-contrast light microscopy and SEM), plasma membrane integrity (PMI) (using a hypo-osmotic swelling test [HOST]) and SYBR-14/propidium iodide [PI], plasma membrane stability (PMS) (using Annexin-V/PI) and deoxyribonucleic acid (DNA) integrity (using SCSA and flow cytometry [FCM]). The average ejaculate volume was about 3.0–4.0 mL, with good viability (PMI measured by the HOST) and motility (>65% and >70%, respectively). Sperm concentration ranged from 1.1 to 1.2 billion/mL, being also affected by bull age. Whereas semen quality (including sperm output, pH and initial sperm motility) did not differ between the seasons. Few spermatozoa (<15%/ejaculate) had abnormal morphology with abnormalities resembling those in other bovidae. In FT semen, PMI (using SYBR-14/PI) and PMS were highest in winter. Across seasons, ~50% of post-thaw spermatozoa depicted linear motility, a proportion that decreased to ~35% during incubation (38°C for 60 minutes), without marking any seasonal difference. The sperm DNA was hardly damaged (with <3% fragmentation, expressed as DNA fragmentation index [DFI]), among seasons.

Key words: Swamp buffalo, Ejaculate volume, Sperm motility, Sperm morphology.

INTRODUCTION - In Thailand, the vast majority of buffaloes are of the swamp type, which breed is distributed in all parts of the country. They are commonly used as draught animals and their manure is used as fertilizer for rice and other crop cultivation but people also take full advantage of them for transportation, sport (buffalo racing), as a saving bank against hard-time and subsidiary labour for the villagers. For the past decade, the Thai swamp buffalo population has, however, declined rapidly, from 4.2 million head in 1994 to 1.6 million head by 2005, while the number of beef and dairy cattle has increased from 7.4 and 0.2 million to 7.8 and 0.5 million head, respectively, during the same period (DLD 2005a; b). There are many reasons behind this dramatic decrease, such as (1) socio-economic factors including lack of family labour and market price discrimination; (2) government policies concerning issues such as mechanization or modernization of agricultural production; (3) factors such as lack of strong farmer cooperatives, an inefficient livestock marketing system or absence of effective livestock husbandry services;
and (4) technical constraints including lack of breeding bulls as a consequence of males being sold before they reach sexual maturity or the castration of mature bulls to be used as draught animals; as well as low calving rates due to poor management and insufficient feeding (Chantalakhana 2001).

In Thailand, AI was introduced in 1956, and frozen semen doses from Thai artificial insemination (AI) swamp buffalo bulls which are routinely assessed and approved for processing (i.e. extension and freezing) are based on the percentage of sperm motility and sperm concentration have been distributed for AI throughout the country since 1978. However, new methods are nowadays available and their application is the core of this presentation.

Collection and characteristics of Thai swamp buffalo semen

Semen collection and evaluation

Semen collection in buffaloes has been mostly performed by way of an artificial vagina (AV) that using of an AV in buffalo bulls is mainly restricted to those sires kept at AI centres, since they need to be well trained to mount a restrained cow or teaser.

• Colour and density - The colour and density of Thai swamp buffalo semen showed milky to cream in colour with thin to very dense in density (Kunavongkrit and Bodhipaksha 1978; Koonjaenak et al., 2007a). Koonjaenak et al., (2007a) found significant differences in colour and density of buffalo semen were affected by bull age , with an increase in both with age.

• Volume and pH- The average ejaculates volume of mature Thai swamp buffalo is about 3-4 mL per ejaculate with an average pH of 6.9–7.0 (Sukhato et al., 1988; Koonjaenak et al., 2007a). While, the ejaculated volume varies depending on age of buffalo bull with increasing with age in swamp buffalo and also in riverine buffalo (Pant et al., 2003).

• Sperm motility and viability - A mass activity from the adult healthy swamp buffalo shown quick to very quick waves of mass activity, while the average percentage of initial progressive motile spermatozoa range from, 65% to 80% with depending on the age of the sires (Sukhato et al., 1988; Koonjaenak et al., 2007a). Viability of swamp buffalo spermatozooa was reported about 65-75 % which was assessed by using using eosin-nigrosin (Nordin et al., 1990) and/or hypo-osmotict swelling test (HOST) (Koonjaenak et al., 2007a).

• Sperm concentration - Sperm concentration in Thai swamp buffalo bulls have a ranges of sperm concentration of mature swamp buffalo bull about 800 to 1,200 million spermatozoa per mL (spz/mL) (Kunavongkrit and Bodhipaksha 1978; Koonjaenak et al., 2007a) which similar results were observed in riverine buffalo bull (Pant et al., 2003), being older bulls having a higher sperm concentration (Nordin et al., 1990; Koonjaenak et al., 2007a).

• Sperm morphology - The overall total mean percentage of sperm abnormalities of Thai swamp buffalo bull spermatozooa was <15% (Sukhato et al., 1988; Koonjaenak et al., 2007b) that was similar to riverine buffalo (Ahmad et al., 1987). The most common sperm abnormality was a pear-shaped head, followed by knobbed acrosomes, proximal cytoplasmic droplets, simple bent tails, and coiled tails under the head which detected by using phase-contrast light microscopy and also SEM (Koonjaenak et al.,2007b). Moreover, the age of swamp buffalo bull had a significant effect on the incidence of total pathological head shapes, acrosome defects, proximal cytoplasmic droplets, and total tail defects that similar to early reported in buffalo bull (Pant 2000).
Semen quality of Frozen thawed of Thai swamp buffalo

The semen of Thai swamp buffalo bulls used for AI was routinely diluted, in one step, in Tris (hydroxymethyl) aminomethane (Tris)-egg yolk extender plus 8% glycerol, to a final concentration of 120 x 10^6 spz/mL and then, the extended semen was packed into 0.25 mL plastic straws, each containing ~30 x 10^6 spermatozoa, and frozen using a programmable biological freezer (Koonjaenak et al., 2007d). The frozen straws were stored in liquid nitrogen (–196°C) until distributed for AI throughout the country.

Characteristics of thawed spermatozoa of Thai swamp buffalo bull

Several parameters are used to determine the quality of spermatozoa after frozen-thawed (FT), particularly motility, forward motility, plasma membrane integrity (PMI), plasma membrane stability (PMS) and also sperm chromatin stability. The percentage of progressive sperm motility of frozen-thawed Thai swamp buffalo semen under light microscope with phase contrast was about 40-45 % (Sukhato et al., 1988; Koonjaenak et al., 2007a), which is likewise with the study of Koonjaenak (2007d), an average linear motility using computer-assisted sperm analysis (CASA). Moreover, Thai swamp buffalo spermatozoa still survived at 38 °C for 60 minutes with decrease in linear motility about 10-15 % (Koonjaenak et al., 2007d). This figures is consistent with early studies in riverine buffalo as Murrah buffalo (Narasimha Rao et al., 1986).

Koonjaenak (2007d) reported the PMI and PMS of FT spermatozoa of Thai swamp buffalo that was assessed using SYBR-14/Propidium iodide (PI) and Annexin-V/PI with flow cytometry (FCM), both sperm attributes, which are of utmost concern for the fertilizing ability of a spermatozoon, appeared significantly (as proportions of spermatozoa per frozen-thawed sample) when the semen was collected/processed in winter (55–56%), and were lowest during the rainy season (40–43%). However, the value for PMI results in those study are in agreement with previous studies using hypo-osmotic swelling (HOST) assays in Murrah buffalo (Shukla and Misra 2006), but are higher than the value reported for Nili-Ravi buffalo (Rasul et al., 2001). And also PMS of FT spermatozoa of Thai swamp buffalo was close to earlier observations in B. taurus using the same method (Anzar et al., 2002; Januskauskas et al., 2003). While, the sperm DNA was hardly damaged using sperm chromatin structure assay (SCSA), with average <3% fragmentation among seasons, (expressed as DNA fragmentation index [DFI]), being best during the rainy season, although this variable was positively related to loose abnormal sperm heads (Koonjaenak et al., 2007c).

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