Research Report

Effect of incorrect storage of bull semen samples on sperm morphology assessment

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Abstract

Thirteen pairs of bull semen samples were assessed. One of each pair of samples was inadvertently stored in phosphate buffered saline (PBS) that was used for analysis of motility immediately after collection. Other sample of the pair that was correctly stored in buffered formol saline (BFS) was assessed later. Proportion of morphologically normal sperm was different ($p = 0.0021$) between the 2 storage methods. Most of the abnormalities in the PBS samples were loose and detached heads. Closer examination of these sperm illustrated changes in the composition of the tail, with an apparent loss of the plasma membrane. This serendipitous error allowed documentation of the importance of correct storage of semen samples for morphological assessment of sperm, and the tertiary defects detected with the incorrect storage of semen samples in semen extender. Tertiary defects should always be considered a possibility during morphological assessment of sperm via spermiogram.

Keywords: Bull sperm, tertiary morphological abnormalities, detached heads

Introduction

Fertility has been defined as the ability to produce young, that for a male means there is a requirement to produce viable sperm. Assessment of the quality of the ejaculate from a male animal requires examination of a semen sample via spermiogram. Color of the ejaculate, and sperm motility and morphology are assessed to identify the likelihood of bulls that are infertile or subfertile. Sperm morphology is arguably the most important criterion for assessing fertility, with teratozoospermia an often under-rated aspect of subfertility or compromised fertility.[1] Features determining semen quality are interrelated to at least some degree.[2] Sperm morphology are classified in several ways: 1. primary, secondary or tertiary classification based on the origin of the lesion (testis, epididymis or handling/processing respectively), 2. major or minor abnormalities based on the actual or perceived detrimental effects on fertility, 3. anatomical location of the lesion on the sperm (head, midpiece, tail), and 4. compensable or noncompensable defects, depending on the effect of the relative number of sperm required in the breeding dose.[1,2]

We report observing teratozoospermia because of inadvertent incorrect storage (phosphate buffered saline [PBS]) of semen samples. Morphological defects were classified anatomically, with most samples initially submitted having a large proportion of morphologically abnormal sperm. Aim of this report is to illustrate the differences, and to highlight the importance of, correct storage (in 10% buffered formal saline [BFS]).

Materials and methods

Semen was collected from clinical cases (Animal Care and Ethics Committee approval was not required) via electroejaculation, and samples (for sperm morphological assessment) were submitted in 1.5 ml snap top Eppendorf tubes containing 1.0 ml diluent. Samples initially assessed were submitted in diluent consisting of isotonic PBS that was used to assess bull-side motility. These samples were prepared 10 days after collection, and microscopically assessed 11 and 12 days after collection. The correct samples, consisting of 200 µl of raw semen collected onsite and placed in 1 ml 10% BFS, were submitted and assessed 2 months after collection.

At the laboratory, the contents of the Eppendorf tubes were gently agitated, and 20 µl of the Eppendorf tube contents were placed on a 25 x 75 mm microscope slide (Westlab 25 x 75 x1 mm; www.westlab.com.au). A 22 x 40 mm cover slip

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(Menzel-Glasser; Deckglasser 22 x 40 # 1) was placed over the drop and left overnight to settle. Sperm on the slide were evaluated morphologically using oil immersion 1000 x differential interference microscopy (Nikon Eclipse 80i). Sperm morphology was assessed, and the anatomical abnormalities were recorded. One hundred sperm were counted and classified per slide.

Data were analysed by comparing the median percent normal morphology in each group using paired Wilcoxon test and p value was set at ≤ 0.05 for significance.

Results

Thirteen pairs of samples were submitted and analysed after the initial submission error. Comparison of the 13 paired PBS and BFS samples revealed substantial differences in sperm morphology between the storage methods. For good fertility there should be a minimum of 70% morphologically normal sperm in an ejaculate, with not > 20% having nuclear abnormalities or proximal droplets, and not > 25% acrosomal or tail abnormalities.[3] BFS samples had 12/13 with ≥ 68% morphologically normal sperm and 1/13 of the PBS samples had ≥ 68% normal sperm with 5/13 having < 20% normal sperm. There were 2 samples in the BFS cohort that had ≥ 19% loose or detached heads compared to 12 in the PBS cohort that had ≥ 35% (7 of them had ≥ 55% loose or detached heads [Table 1]). Most abnormalities recorded in the PBS samples were a combination of loose and detached heads. There were differences (p = 0.0021) in the percentage of morphologically normal sperm between (Figure 1) PBS and BFS groups.

Most of the abnormal sperm were classified as either loose (Figure 2 A-B; b.) or detached heads (Figure 2 A-B; a.). A substantial proportion of remaining tail portions (Figure 2 A-B; c.) were abnormal. They were not recorded as such, as they had already been recorded as detached heads.

Discussion

Bull breeding soundness examination has evolved from the origins of what is now the Society for Theriogenology, with the intention of determining the likelihood of infertile and subfertile bulls. Male fertility is determined by spermiogram assessment, with sperm morphology as the prime indicator of male fertility[4] and is correlated to sperm DNA damage.[5] Unacceptable sperm morphology has been the most common reason cited for not having bulls classified as satisfactory breeding animals.[6]

Table 1. Proportion of normal and abnormal (tail, midpiece, and head abnormalities) sperm in the samples of ejaculates stored in phosphate buffered saline (PBS) or buffered formol saline (BFS)

Sample number	Percent normal	Percent abnormal tail	Percent abnormal midpiece	Percent abnormal head	Percent loose/ detached head
1 PBS	21	6	1	0	72
1 BFS	82	0	11	1	6
2 PBS	58	1	2	0	39
2 BFS	82	0	7	0	11
3 PBS	63	2	0	0	35
3 BFS	75	0	2	0	23
4 PBS	2	32	1	0	65
4 BFS	81	2	6	1	10
5 PBS	69	0	3	0	28
5 BFS	68	0	13	0	19
6 PBS	39	2	3	0	56
6 BFS	86	0	3	0	11
7 PBS	19	4	2	0	75
7 BFS	83	0	8	0	9
8 PBS	61	2	2	0	35
8 BFS	85	0	2	1	12
9 PBS	49	4	5	0	42
9 BFS	89	0	7	1	3
10 PBS	9	4	1	0	86
10 BFS	76	0	9	1	14
11 PBS	0	0	0	0	100
11 BFS	84	0	2	1	13
12 PBS	62	0	0	0	38
12 BFS	89	0	5	3	3
13 PBS	0	7	1	0	92
13 BFS	41	2	37	13	7

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There were a substantial number of detached heads in most of the original (PBS) samples that were assessed from this cohort of bull ejaculates (Table 1). Reasons for detached heads (a small proportion [5.1 ± 0.74%] is acceptable) include: bulls with testicular hypoplasia (implicated as a hereditary trait in at least some animals), transient phenomenon in some bulls with testicular degeneration or other inflammatory conditions, and conditions associated with testicular hyperthermia.[2] A relatively commonly reported reason for detached heads is senescence as a result of duct stasis.[2]

Other reported defects manifesting as detached heads include the stump tail sperm defect that on closer examination include a rudimentary tail or short stump as a tail. Decapitated sperm defect that typically has > 80% of the sperm affected with most part of tails motile and have a characteristic loop in the midpiece.[7]

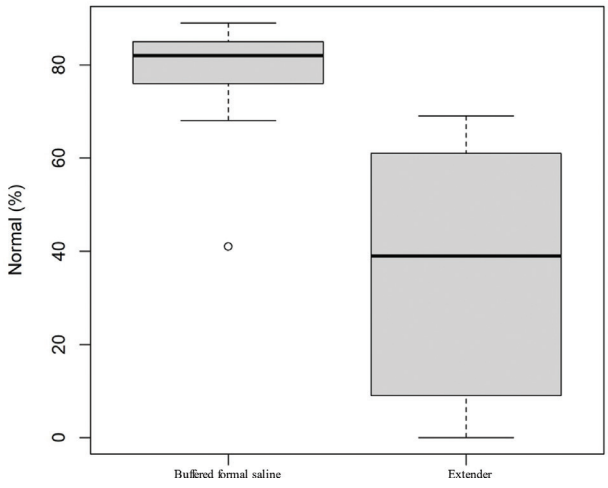

Figure 1. Box and whisker plot of the proportion (%) of normal sperm for each of the treatment groups: buffered formal saline (BFS) and extender (PBS); dark line is the median, upper and lower box limits represent the upper and lower quartiles; whisker lines represent the lower and upper data extremes and circles represent individual outlier data points.

In the context of this report (Figure 2 A-B; b, c) the short tail sperm defect that has been reported in pigs and anecdotally in cattle[7] could be considered a possibility. Tails in these samples were not short and the condition is unlikely to occur in such a high proportion of submitted samples.

Sperm tails were often not critically assessed in the initial examination in these samples as a result of them being detached or loose and were documented as such in the morphological assessment. It illustrates that close examination of the tail, and their attachments to the head were abnormal, with the plasmalemma of the tail being obviously abnormal, probably as a result of bacterial consumption of the phospholipids.[8]

During sperm morphological assessment, some aberrant movement of the material on the PBS slides was detected and noted. Retrospectively, this is abnormal in BFS preserved samples, and is likely because of the presence of motile bacteria or other microorganisms. Agglutination of sperm preserved in BFS can be ameliorated by the use of formol citrate.[3]

Tertiary defects are often referred to in texts associated with assessing a spermiogram. They are however, unlikely to have any effect on the fertility of the bull, resulting in diagnostic errors and incorrect decisions on the outcome of the animals concerned. In particular, poor handling and the use of inappropriate extender or in this case preservative, can result in the incorrect diagnosis due to these tertiary defects (Table 2).[8]

Conclusion

Inadvertent error of assessing incorrectly processed semen samples has allowed for a serendipitous opportunity to report abnormalities that are most likely due to incorrect storage of samples submitted for morphological assessment of an ejaculate. It is clear from these observations that appropriate collection and storage of samples for morphological assessment is required when assessing sperm morphology. Incorrect sample preparation and storage should be considered as a reason for an abnormal spermiogram, especially when a large proportion of detached heads, with or without tail and midpiece plasma membrane abnormalities/deficits, are detected in a semen sample.[8]

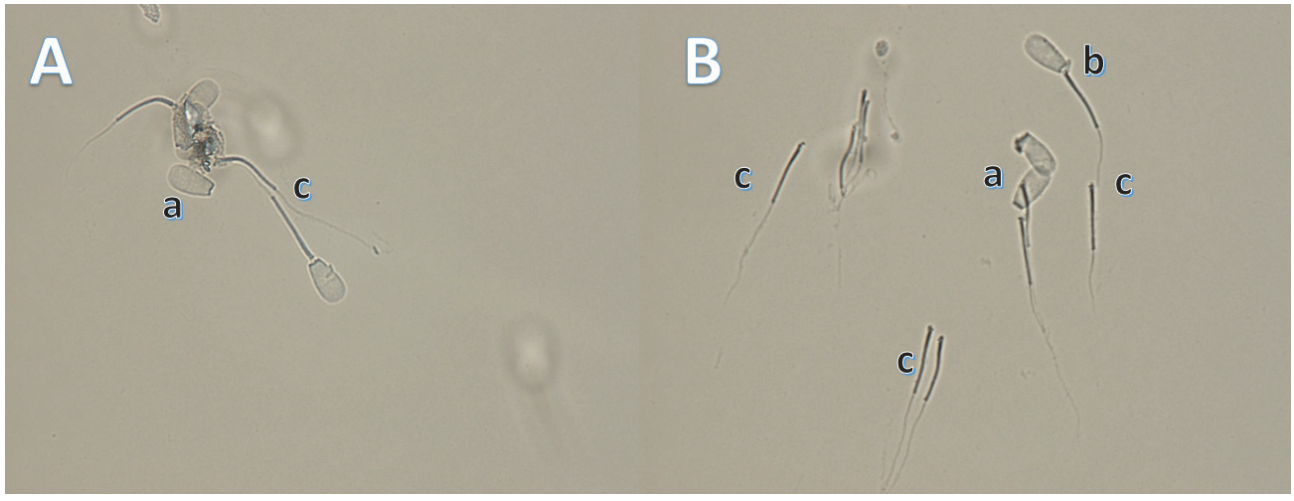

Figure 2. A-B. Photomicrographs (differential interference contrast microscopy x 1000) of sperm from semen samples submitted in semen extender (PBS); a. detached heads, b. loose head, and c. detached tails without plasma membranes.

Table 2. Abnormal spermiogram findings (tertiary defects) because of sample preparation error is illustrated (reproduced[8] with permission)

8.8.11 Defects caused by sample preparation

Several sperm problems, including those related to movement and morphology, can be caused by environmental factors which occur after the sample is collected that can lead to errors in diagnosis and prognosis.

Common problems listed in this category are listed below:

Sample preparation error	Indicators in sperm
Cold Shock	• Observation of particular sperm movements, such as moving backwards, circling and 'shimmering' in place.
	• Suspiciously large difference between motility and live/dead assessments (especially if sperm morphology is otherwise good).
	• Increased distal midpiece reflexes without accompanying retained droplets
Poor handling (temperature, contamination, rough handling, inappropriate extender)	• Decreased percent intact acrosomes
	• Increased loose/degenerating acrosomes
	• Presence of evident numerous bacteria
	• Excessive clumping
	• Increased bent and coiled tails
	• Increased loose/detached sperm heads
	• Evidence of crystal formation
Nonisotonic media	• Increased distal midpiece reflexes and tail abnormalities
Poor preparation/staining/microscopy	• Sperm stained too dark, too light
	• Sperm too concentrated or too sparse on the slide
	• 'Halo' effect and 'cracks' in the stain monolayer
	• Undetected subtle sperm defects (especially if consistent)
	• Curiously increased numbers of narrow heads
Excessive motion or torsion (coverslip)	• Increased loose/detached sperm heads
	• Evidence of disruption (broken midpieces and tails, sperm debris) of the sperm preparation

p. 92 examination of sperm - bull breeding soundness evaluation.

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Conflict of interest

None to report and no funding is associated.

Author contributions

Authors conceived the idea, AG wrote substantial part of the manuscript, MB reviewed it, and both authors approved submission.

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