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Sperm Morphology and Fertility of Progeny-Tested AI Swedish Dairy Bulls

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Abstract: Use of bull semen with high levels of sperm abnormalities, reflecting genital dysfunction, is not recommendable to use for AI since it most likely leads to sub-fertility. Sperm morphology deteriorates with age, a source of concern when using ageing progeny-tested AI-bull sires. However, whether a relationship between sperm morphology and fertility after AI in progeny-tested bull sires is present remains to be proven and constituted the reason for a retrospective study of progeny-tested bull sires of the Swedish Red and White (SRB) and Swedish Holstein (SLB) breeds performed with data of 8 SRB and 4 SLB sires analysed in an University specialized andrological laboratory. Particular attention was paid to the influence of age and breed as well as the variation between- and within-bull for presence and level of sperm abnormalities. Sperm morphology differed between sires, ejaculates and breed, with 6/12 sires having ejaculates with more than 10% of morphologically deviating sperm heads, a commonly used threshold for young AI bulls. However, with the exception of pear-shaped or narrow at the base anomalies, individual defect mean values were always within what is expected for a bull sire and considered within acceptable limits. Breed and age affected some semen (ejaculate volume) and sperm morphology variables (nuclear pouches, acrosome defects and head contour). Estimated breeding values for male fertility output differed significantly among bulls, with the percentage of morphologically normal spermatozoa in the overall ejaculate being positively related to fertility. Few individual sperm abnormalities correlated negatively to fertility, as pear-shaped sperm heads, proximal droplets or loose heads (SRB), probably owing to the reduced sire material examined. In conclusion, the presence of relationships between sperm morphology and fertility after AI calls for routine, frequently done assessments of sperm morphology in AI-stud bull sires during their entire active life.

Key words: Sperm abnormalities, NRR, fertility estimates, SRB, SLB, bull

INTRODUCTION

Artificial Insemination (AI) has been the reproductive biotechnology that has made possible the safe use of semen from selected sires on a breeding female population, thus preventing the dissemination of venereal diseases. Furthermore, application of AI as a tool for dissemination of semen from sires with characters of importance has contributed to the improvement of the genetic quality of breeding herds. This improvement has been exponential in dairy cattle where use of frozen semen for AI is most common, providing possibilities for the commercial dissemination of genetic material all over the world (Thibier and Wagner, 2000). A pre-requisite for the best use of this genetic material is to obtain acceptable fertility after AI. For this reason, both screening of the normality of the semen and the correct evaluation of bull fertility are essential to the AI industry. At present, the most common means for estimating bull fertility are Non-Return Rates (NRRs), i.e., the percentage of cows not returning to oestrus at some (most often 56 days) interval post-AI. Such estimate is indirect but rapid and it is therefore used most often when a population of females is under a certain degree of control, for instance when cows are enrolled in a milk-recording programme. However, such estimates are under the influence of several factors that can overestimate the outcome. Examples of these factors are the reliability of the oestrus

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control systems, the season of the year, the category of the females inseminated, the area, the inseminator, etc. Non-return rates are therefore, often statistically corrected for the influence of these factors and thus named "corrected" NRRs, or estimated breeding value (Stålhammar *et al*., 1994). In most breeding programmes, semen from young bulls is collected, tested for normality and thereafter processed to produce AI-doses which are inseminated on a proportion of the breeding population to determine its fertility and lay the basis for the progeny testing procedure. The progeny testing is a procedure that takes 3-4 years and includes analyses of the collected data using a Best Linear Unbiased Prediction (BLUP) system, a procedure that estimates breeding values of a sire based on the phenotypic performance of himself and his daughters (Werf, 2000). During this 3-4 year waiting period, the bulls are reared indoors, in groups of 8-12 sires at the bull station. Once the results of the progeny testing, based on the results from their daughters´ first lactation are known, the top-ranked bulls return to the semen production scheme and are used as proven sires (élite bulls). Under these circumstances, a progeny tested bull has reached the age of 4.5-5 years and qualitative and/or quantitative changes could have occurred in his semen so that the fertility level assessed from 56d-NRR when the bull was young may no longer be valid.

Current semen (ejaculate) evaluation is done immediately after collection and comprises the determination of aspect, volume (also by weight) and sperm concentration. As well, the subjective assessment of sperm motility and obvious deviations in sperm morphology (bent tails, proximal droplets etc) are done pertaining the indirect measure of sperm viability and normality prior to processing. Motility assessments are also routine post-thaw, with thresholds for use or refusal of the processed semen. Seldom is the semen of the sires assessed for sperm morphology, an evaluation being done when suspicions of pathologies that could compromise sperm production or function exist. The presence of a large number of sperm abnormalities in the semen is not only indicative of pathological processes in the testes, epididymis or accessory sexual glands (Lagerlöf, 1934), but also associated with a decreased fertility of the semen. On the other hand, use of semen morphology as a measurement of semen fertility (or sire fertility) has been discussed for its value when semen within normal values is assessed (Rodriguez-Martinez, 2003). Sperm quality differs with the age of the sires. Considering that AI-dairy bulls are tested for fertility at an early age (usually 11-13 months of age), thresholds for sperm normality in terms of sperm morphology have been studied and set (Söderquist *et al*., 1991). Oten, these thresholds are also used for bulls of an older age, including proven bulls. Recently, Hallap *et al*. (2004) studied sperm morphology post-thaw in AI bulls, both when young (11-13 mo) and while bulls were awaiting progeny testing (4 y), finding that while sperm motility and membrane integrity improved when sires were older, sperm morphology did not significantly change with age. Since the genital normality of the bulls can change over time and thus affect the quality of the semen they produce, there is a logical need to determine which levels of sperm abnormalities proven bulls have and also if these levels of abnormalities could be related to the fertility of the sires. The aim of the present study was to analyse the relationship between sperm morphology in the semen of proven (e.g., progeny tested) bull sires of the Swedish Red and White (SRB) and Swedish Holstein (SLB) breeds to be used for the production of frozen doses for Artificial Insemination (AI) and their fertility after AI. Particular attention was paid to the influence of age, breed and the variation between- and within-bull for presence and level of sperm abnormalities.

MATERIALS AND METHODS

Source of semen: Semen collected from 8 Swedish Red and White (SRB) and four Swedish Holstein (SLB) proven bulls, e.g., selected for the breeding programme after a period of fertility assessment and progeny testing, was used in the present study. The bulls were stationed at Svensk Avel ek för (Örnsro, Skara) where the collected semen (sampled via artificial vagina) was routinely checked for volume (by weight determination) and sperm concentration (using a photometer). Sperm motility was subjectively estimated under a phase contrast microscope (200x) equipped with a warm stage (+38°C), by the same operator. The mean value from evaluations on four fields was recorded. At regular intervals (1-3 months), samples from two consecutive ejaculates were prepared and sent to a specialized andrological laboratory, that acts as the reference laboratory for Sweden, located at the Division of Comparative Reproduction, Obstetrics and Udder Health, Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, SLU, Uppsala, for manual determination of sperm concentration and detailed morphological examination of sperm abnormalities and presence of foreign cells. Aliquots of the semen to be examined were used to prepare thin- and thick, escalated smears that, after being allowed to air-dry, were packed and sent. As well, aliquots of the semen were fixed in buffered formaldehyde (Hancock, 1957) for the preparation of wet smears. Finally, a part of the raw semen was used to determine sperm concentration, being assessed in two separate counting chambers using a

Bürker haemocytometer, as initially described by Bane (1952). This regular assessment forms part of the quality assurance system of Svensk Avel ek för by agreement with SLU. Semen was collected from the bulls when they were between 60-106 months of age and the total number of evaluated ejaculates for these 12 bulls was 107.

Morphological examination of spermatozoa: Sperm head morphology was studied in thin smears prepared and stained with Carbol-fuchsin according to the method described by Williams (1920) and modified by Lagerlöf (1934). Five hundred spermatozoa were counted in each smear at a magnification of 1,000x in a light microscope to depict presence of abnormal head shapes (pear-shaped, narrow, giant, small and abaxial heads, detached heads [those with normal and abnormal head morphology] as well as abnormal mid-pieces). The presence of proximal cytoplasmic droplets, abnormal acrosomes, detached heads and abnormalities of the mid-piece and the tail were studied in wet preparations made from formol-saline fixed samples (Hancock, 1957) under a phase-contrast microscope at a magnification of 1,000x. Two hundred spermatozoa were counted in each preparation and the abnormalities (acrosomes, nuclear pouches, proximal and distal cytoplasmatic droplets, mid-pieces and abnormal tails (double folded, single bent and coiled tails) were classified according to a system developed by Bane (1961). The number of spermatozoa showing each class of abnormality was expressed as a percentage of the counted spermatozoa. A thick, dried smear stained with haematoxilin-eosin or Papanicolaou was used to determine the presence and relative quantity of foreign cells (such as cells of the seminiferous epithelium, epididymal cells, epithelium of the urethra, prepuce/penis, accessory glands) and inflammatory cells (leukocytes, lymphocytes, monocytes/macrophages). The relative presence of each foreign cell type were as 0 = absent, 1 = scarce, 2 = rich, 3 = very rich.

Fertility measures: The fertility of the proven bulls was reported, for each freezing batch prepared from the examined ejaculates, by Svensk Avel ek för as the non-corrected percentages of 56 day non-return rates (56d-NRR) (i.e., the percentage of inseminated females not returned to service within 56 days after AI with their frozen-thawed semen) or as estimated breeding value, e.g., values which, based on the 56-d NRR percentages, were corrected for a series of factors influencing fertility in cattle (season, inseminator, category of female, location of the country, etc). Both values had been used as a measure of fertility of individual freezing batches of the evaluated semen samples/bulls and compared.

Statistical analysis: The information from the 107 semen samples (sperm production, sperm morphology and fertility) was analysed statistically by the Statistical Analysis Systems package (SAS Institute Inc., Cary, NC, USA, version 8). Analysis of variance (PROC GLM) was applied to the data, according to a statistical model including the fixed effects of breed (SLB and SRB), age at semen collection (3 groups, < 70 months, 79-79 months and >80 months of age) and bull nested within breed (4 SLB; 48 SRB). Residuals were calculated and these residuals were correlated (using Spearman correlation) within breed. These residuals were also used for calculating within bull-variation (SD), as well as means for each bull. These means and SD were used for calculating correlations on bull-level. Differences were significant ($p<0.05$).

RESULTS

The progeny-tested sires were examined for sperm morphology when they averaged 76.9±8.8 months of age. The characteristics of the collected ejaculates are presented in Table 1. Semen volume averaged 6.0±0.8 mL, with an overall mean total sperm number of 7.4±1.6 billion

Table 1: Age interval during collections, number of ejaculates, volume of ejaculate, sperm concentration (per mL), Total Sperm Number (TSN) per ejaculate and of sperm motility (initial and post-thaw) among evaluated sires (SRB: Swedish Red and White Breed, SLB: Swedish Holstein Breed, Mean±SD)

					Sperm numbers (10^9)		Sperm motility (%)	
Breed	Bull	Bull age during evaluation (mo)	Ejaculates (n)	Volume (mL)	per mL	TSN	Initial	Post-thaw
SRB	A	66.2±5.3	7	7.2±2.4	0.77±0.2	5.9±3.1	71.8±2.4	51.9±2.4
	B	78.0±7.6	11	6.9±2.0	1.3±0.2	9.3±3.7	72.5±1.9	53.1±2.2
	C	89.0±7.2	12	6.5±2.2	0.99±0.1	6.4±2.3	72.5±2.6	52.1±1.7
	D	96.0±8.8	7	5.3±0.9	1.40±0.3	8.1±1.3	72.5±2.0	53.7±2.5
	E	76.2±7.1	7	6.2±1.2	1.12±0.3	6.9±1.9	71.8±2.4	53.1±2.4
	F	69.7±6.1	4	5.4±0.9	1.30±0.3	7.4±1.9	73.1±2.4	55.0±0.0
	G	68.0±5.5	9	4.8±1.1	1.07±0.2	5.2±1.9	71.9±1.7	55.8±3.7
	H	73.2 ±6.8	14	6.1±2.1	1.20±0.4	7.7±4.2	70.0±3.8	49.1±10.4
SLB	I	70.6±7.5	14	7.0±2.2	1.30±0.4	9.5±5.0	73.2±2.5	51.9±6.9
	J	82.0±7.5	11	6.0±1.3	0.94±0.1	5.7±1.4	70.9±3.2	51.8±2.4
	K	77.0±6.8	5	4.9±0.9	1.40±0.3	6.9±2.0	72.0±2.1	50.8±1.4
	L	77.0±8.3	6	6.8±1.1	1.50±0.2	10.3±2.1	71.7±2.0	52.5±2.5
Total means±SD		77.0±7.0	107	6.0±1.5	1.2±0.3	8.4±3.4	72.0±2.4	52.6±2.4

Table 2: Sperm morphology (%) of proven sires (A-L) of the Swedish Red and White (SRB) or Swedish Holstein (SLB) breed (mean±SD)

		BULLS												
		SRB								SLB				
Sperm abnormality		A	B	C	D	E	F	G	H	I	J	K	L	Average all bulls
Head	Pear shaped	7.6±2.7	6.5±2.5	6.1±1.6	2.9±1.1	1.2±0.6	2.5±1.9	2.3±1.5	20.7±5.5	4.1±1.8	2.1±1.2	1.8±0.8	1.8±0.8	6.5±6.6
	Narrow base	6.7±2.5	3.3±1.4	1.8±0.9	3.0±1.0	1.8±0.8	2.1±1.6	0.6±0.5	8.1±3.1	8.0±1.6	1.9±0.6	1.9±0.9	1.9±0.9	4.3±3.4
	Narrow	0.8±0.5	0.2±0.2	0.1±0.2	0.4±0.4	0.6±0.5	0.5±0.7	0.3±0.2	0.2±0.2	0.4±0.3	1.0±0.5	0.5±0.6	0.5±0.6	0.4±0.4
	Abn contour	0.6±0.5	0.4±0.2	0.4±0.4	0.2±0.3	0.3±0.3	0.3±0.2	0.4±0.3	0.3±0.3	0.2±0.2	0.4±0.5	0.3±0.4	0.3±0.4	0.3±0.3
	Variable size	1.1±0.6	1.9±0.7	2.6±1.3	0.7±0.8	1.3±0.7	1.2±0.4	1.9±0.7	1.6±0.8	0.6±0.3	1.4±0.8	0.9±0.8	0.9±0.8	1.5±0.9
	Loose abnormal	4.7±3.2	0.6±0.5	0.7±0.7	0.2±0.2	0.2±0.3	0.4±0.4	0.2±0.2	4.8±1.9	0.1±0.2	0.4±0.3	0.2±0.2	0.2±0.2	1.3±2.8
	Un Developed	1.1±0.9	0.9±0.4	1.7±1.1	0.4±0.4	0.2±0.2	1.0±0.4	1.1±0.4	0.9±0.4	0.4±0.4	0.5±0.7	0.4±0.3	0.4±0.3	0.8±0.7
	Nuclear pouches	0.4±0.5	0.1±0.2	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.1±0.2	0.9±0.9	0.0±0.0	0.1±0.2	0.0±0.0	0.0±0.0	0.2±0.4
	Acros defect	0.2±0.3	0.2±0.2	0.1±0.1	0.0±0.0	0.2±0.4	0.4±0.4	0.1±0.1	0.5 ±0.7	0.2±0.2	0.1±0.2	0.0±0.0	0.0±0.0	0.2±0.3
	Acros Abnormal	2.6±2.2	1.9±1.1	2.0±1.4	1.4±0.9	1.3±0.9	2.1±0.7	2.4±1.7	6.0±2.5	1.6±1.9	2.4±1.7	3.2±2.1	3.2±2.1	2.6±2.1
	Abaxial	0.1±0.2	0.1±0.2	0.1±0.2	0.1±0.2	0.1±0.2	0.1±0.1	0.1±0.2	0.1±0.1	0.1±0.1	0.4±0.3	0.1±0.2	0.1±0.2	0.1±0.2
	Total Path.heads	22.6±7.6	14.0±4.2	13.4±4.0	8.0±3.2	5.7±1.3	7.9±4.7	6.9±2.7	36.7±7.9	14.0±2.6	8.1±3.3	20.2±6.4	6.3±2.3	15.0±11.0
Neck	Loose heads	8.3±5.8	1.2±1.0	1.2±1.0	0.8±0.8	1.6±1.2	2.1±1.9	0.8±0.5	5.8±2.4	0.4±0.7	1.2±1.4	1.3±1.0	1.3±1.0	2.4±3.1
Cyt Drops	Proximal	1.7±0.5	1.5±0.9	1.0±0.9	2.2±0.9	0.6±0.6	2.4±0.5	2.2±0.9	8.8±4.9	0.3±0.4	0.9±0.8	5.3±6.3	5.3±6.3	2.5±3.6
	Distal	17.5±11.5	4.0±3.4	9.7±7.3	2.6±1.6	5.9±6.8	4.3±6.2	6.9±6.9	10.4±7.0	0.8±0.6	5.1±4.0	13.0±14.0	13.0±14.0	6.6±7.5
Mid. Piece	Abn.midpiece	0.8±0.9	0.6±0.5	0.9±0.7	0.4±0.4	0.8±0.9	2.0±0.9	0.7±0.7	1.1±0.8	0.9±0.8	1.2±0.9	0.9±0.4	0.9±0.4	0.9±0.7
Tail	Simple bent	2.0±2.4	2.8±3.1	2.3±1.5	0.3±0.3	0.9±0.7	0.5±0.5	0.3±0.2	5.5±3.3	1.0±0.8	1.2±0.6	2.0±1.7	2.0±1.7	1.7±1.5
	Under head	0.9±0.9	0.9±0.7	1.3±0.9	0.2±0.3	0.1±0.2	0.4±0.2	0.3±0.3	1.6±1.2	0.3±0.4	0.6±0.5	0.7±0.4	0.7±0.4	0.7±0.8
	Double-folded	0.4±0.5	0.4±0.4	0.8±0.5	0.1±0.2	0.9±0.4	0.4±0.4	0.3±0.5	0.7 ±0.7	0.1±0.2	1.4±1.4	0.5±0.8	0.5±0.8	0.6±0.7
Total	Normal spz.	67.0±14.1	83.4±5.7	81.2±7.0	92.0±1.9	87.3±6.5	87.3±7.8	86.4±6.1	61.3±13.1	93.8±3.4	85.3±6.7	74.2±19.8	74.2±19.8	82.5±10.0

Table 3: Fertility after artificial insemination with frozen-thawed semen from the proven sires (A-L) expressed either as uncorrected percentages of 56-day Non-Return Rates (56d-NRR) or corrected for factors influencing outcome (Estimated breeding values). SRB: Swedish red and white breed, SLB: Swedish Holstein Breed, means±SD

Breed	Bull	Uncorrected 56-d NRR (%)	Estimated breeding values
SRB	A	64.0±7.0	98.0±7.0
	B	67.7±2.1	99.4±5.5
	C	64.4±3.9	96.5±4.0
	D	71.7±0.4	106.0±5.5
	E	64.2±3.6	97.2±3.7
	F	68.2±1.1	102.0±6.1
	G	63.8±2.9	97.5±3.0
	H	65.3±3.3	96.8±5.9
SLB	I	69.5±3.7	101.0±4.3
	J	66.0±3.6	96.2±3.2
	K	58.5±5.6	85.8±6.2
	L)	67.7±2.1	99.4±5.5
Average±within bull SD		66.3±4.3	98.2±6.0

spermatozoa. Initial sperm motility averaged 72.0±0.9% while the post-thaw sperm motility of the frozen batches averaged 52.6±1.8%. Significant differences were detected for ejaculate volume ($p<0.05$) total sperm number ($p<0.001$) and sperm concentration ($p<0.05$) within and between breeds but there were no significant differences within or between breeds with regards to initial or post-thaw motility.

Sperm morphology of the examined ejaculates per sire is summarized in Table 2. The overall frequency of spermatozoa depicting normal morphology ascended to 82.5±10.0 %, varying significantly ($p<0.05$) among sires. Overall, the percentage of total sperm head abnormalities showed a mean of 15.0±11.0%, with 6/12 sires having more than 10% of sperm head abnormalities (highest mean was 36.7±7.9%). However, the percentages of individual sperm abnormalities varied largely among bulls and ejaculates. The highest mean value for individual sperm defects within bull was recorded for pear-shaped sperm heads (20.7 %, bull H) with only two bulls having significantly higher ($p<0.05$) values than the rest of the sires. Approximately 75% of the sperm morphology defect categories were less than 2% of all studied spermatozoa, approximately 12 % of the bull means were above 2% but lower than 5%, approximately 9% of the means occurred in between 5 and 10% and few other deviating values (>10%) also recorded represented only 0.04% of the means of sperm morphology categories (Table 2). Differences were found for most sperm abnormalities, but with the exception of pear-shaped or narrow at the base anomaly, the mean values were always within what is expected for a bull sire, considered within acceptable limits.

The relative quantity of foreign cells in the ejaculates examined was low and did not exceed class level 1 (e.g., scarcity).

Table 3 presents the fertility (means±SD) of the freezing batches corresponding to the ejaculates examined for morphology, grouped per bull. Fertility is expressed either as uncorrected percentages of 56d-NRR or as estimated breeding values. While there were no significant differences in fertility among bulls when the 56d-NRR were compared, significant differences ($p<0.001$) were present among bulls for corrected fertility values.

Breed and Bull within breed affected most sperm variables, including both total sperm numbers per ejaculate ($p<0.001$) and the number of pathological sperm heads ($p<0.01$). Most sperm variables varied significantly between ejaculates. Age of the sires affected some semen and sperm parameters, e.g., ejaculate volume ($p<0.05$), post-thaw sperm motility ($p<0.05$) , total sperm number within an ejaculate ($p<0.001$) and proportion of nuclear pouches in spermatozoa ($p<0.05$). While the percentage of morphologically normal spermatozoa in the overall ejaculate set up was positively related to estimated breeding values ($r = 0.7$, $p<0.001$), the relations for individual morphological defects and fertility differed

between breeds. While for SRB bulls the percentage of normal spermatozoa correlated positively with fertility ($r = 0.27$, $p<0.05$), those of pear-shaped sperm heads, loose heads and proximal droplets were negatively related to fertility ($r = -0.80$, $p<0.01$; $r = -0.39$, $p<0.05$ and $r = -0.26$, $p<0.05$, respectively); significant relations were not found for SLB bulls, most likely owing to the low number of SLB bulls examined.

DISCUSSION

The present retrospective study aimed at determining whether sperm morphology in the semen of progeny tested bull sires of the Swedish Red and White (SRB) and Swedish Holstein (SLB) breeds related to the fertility outcome after AI. Sperm morphology differed between ejaculates, sires, breed and age. Six out of the twelve sires had ejaculates with more than 10% of morphologically deviating sperm heads but with the exception of pear-shaped or narrow at the base anomalies, the mean values were always within the levels expected for a bull sire and thus considered within acceptable limits. Statistically corrected fertility output differed significantly among bulls and some sperm abnormalities were clearly, negatively correlated to fertility, in particular the total percentages of morphologically abnormal sperm heads. Few individual sperm morphology variables (defects) only related to fertility, yet being well within the means considered acceptable for bulls.

Significant differences were found between the two breeds with regard to the corrected fertility and morphological defects. No significant difference was found, however, with uncorrected fertility (e.g., 56d-NRR), in contradiction with other reports (Söderquist *et al*., 1991). Such differences can, obviously, reside in the number of the sires and ejaculates explored, the different age of the sires examined, as well as the different seasons when AIs were performed, all of which make it difficult to compare the outcomes of these studies.

Sperm morphology varied significantly with age, in particular the total percentage of morphologically normal spermatozoa and of individual sperm abnormalities (nuclear pouches, acrosome defects, abnormal sperm head contour etc). These findings agree with those of Söderquist *et al*. (1996) when looking at different defects in frozen-thawed semen of younger bulls (aged 14-36 months). Other studies linking sperm morphology and age are a bit contradictory. Foote *et al*. (1977) reported an improvement in sperm morphology when bull sires reached 3-4 years of age while Padrik and Jaakma (2002) have shown a decrease in normal morphology in bulls from 4-5 and 6-7 years of age. The present results appear contradictory to what has been reported earlier in Sweden (Hultnäs, 1959; Söderquist *et al*., 1991) who could not find any relationship between age and morphological characteristics among unselected bulls aged from 15-26 months, or the findings by Hallap *et al*. (2004) that registered no significant difference in sperm morphology in bull sires when aged 1 or 4 years. Again, individual differences and differences in age range can be behind the variations in results encountered. Moreover, ageing is an ongoing process with important individual variation. Bulls should be examined in longitudinal studies from when they start providing semen for AI until they are culled (for age) in order to determine when they attain peak maximal sperm production and when the ageing effect starts.

Percentages of morphologically normal or of abnormal spermatozoa are related, positively or negatively, to conception rate (Jaczewski and Kazimirow, 1997; Gotschall and Mattos, 1997; Fitzpatrick *et al*., 2002; Padrik and Jaakma, 2002).

CONCLUSION

The results from the present study confirm there is a relation between the number of morphologically normal spermatozoa in the ejaculate and the fertility outcome after AI of frozen-thawed semen produced with this particular ejaculate. As well, presence of a certain level of morphologically abnormal spermatozoa negatively affects fertility and the results found in the present study suggest that a level of morphologically abnormal sperm heads above 10% remains a valuable indicator for this relationship. In sum, the results of the present study confirm there is a relationship between sperm morphology and fertility after AI in bull sires thus calling for routine, frequent screenings of sperm morphology in AI-stud bull sires. Owing to the possible presence of a relationship with age, at least in some sires, it is of importance to follow up these evaluations during their entire active life.

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REFERENCES

Bane, A., 1952. A study on the technique of hemocytometric determination of sperm motility and sperm concentration in bull semen. Cornell Vet., 42: 518-531.

Bane, A., 1961. Acrosomal abnormality associated with sterlity in boar. Proc. 4th Int. Congr. Anim. Reprod. A.I., The Hague, IV: 810-817.

Fitzpatrick, L.A., G. Fordyce, M.R. McGowan, J.D. Bertram, V.J. Doogan, J.de. Faveri, R.G. Miller and R.G. Holroyd, 2002. Bull selection and use in northern Australia. Part 2. Semen traits. Anim. Reprod. Sci., 71: 39-49.

Foote, R.H., G.E. Seidel, J. Hahn and W.E. Berndtson, 1977. Seminal quality, spermatozoal output and testicular changes in growing Holstein bulls. J. Dairy Sci., 60: 85-88.

Gotschall, G.S. and R.C. Mattos, 1997. Findings of andrological examinations of Bos taurus and Bos indicus bulls. Revista Brasilieira Reprod. Anim., 21: 25-28.

Hallap, T., M. Håård, U. Jaakma, B. Larsson and H. Rodriguez-Martinez, 2004. Variations in quality of frozen-thawed semen form Swedish and white AI sires at 1and 4 years age. Int. J. Androl., 27: 166-171.

Hancock, J.L., 1957. The morphology of Boar spermatozoa. J.R. Microsc. Soc., 76: 84-97.

Hultnäs, C.A., 1959. Studies on the variation in mating behavior and semen picture in young bulls of Swedish red and white breed and on causes of this variation. Acta Agric. Scand., Suppl. 6, Thesis, Stockholm

Jaczewski S. and E. Kazimirow, 1997. Morphological characters of bull semen and conception rate. Medycyna Weterynaryjna, 33: 561-563.

Lägerlöf, N., 1934. Morphologische Untersuchungen Uber Veränderungen im Spermabild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilität. Acta Pathologica et Microbiologica Scandinavica Suppl. 19, Thesis, Stockholm.

Padrik, P. and U. Jaakma, 2002. Sperm morphology in Estonian Holstein dairy bulls, factors affecting it and relation to fertility. Agraarteadus, 13: 243-256.

Rodriguez-Martinez, H., 2003. Laboratory semen assessment and prediction of fertility: Still utopia? Reprod. Domestic Anim., 38: 312-318.

Söderquist, L., L. Janson, M. Haard and S. Einarsson, 1996. Influence of season, age, breed and some other factors on the variation in sperm morphological abnormalities in Swedish dairy A.I. bulls. Anim. Reprod. Sci., 44: 91-98.

Söderquist, L., L. Janson, K. Larsson and S. Einarsson, 1991. Sperm morphology and fertility in AI bulls. J. Vet. Med., 38: 534-543.

Stålhammar, E.M, L. Janson and J. Philipson, 1994. Genetic studies on fertility in AI bulls. II. Environmental and genetic effects on non-return rates of young bulls. Anim. Reprod. Sci., 34: 13-27.

Thibier, M. and H.G. Wagner, 2000. World statistics for artificial insemination in cattle. Proc 14th ICAR Stockholm 2: 76 (15:2).

Werf, J., 2000. An overview of animal breeding programs. Part1: Introduction and building blocks. Identifying and incorporating genetic markers and major genes in animal breeding programs. University of New England, Armidale, Australia, pp: 1-8.

Williams, W.W., 1920. Technique of collecting semen for laboratory examination with a review of several diseased bulls. Cornell Vet., 10: 87-94.