



# **Sperm morphology in progeny-tested Swedish AI dairy bull sires**

**Anas Al -Makhzoomi**

Master of Science Programme in Veterinary Medicine  
for International Students  
Faculty of Veterinary Medicine and Animal Science  
Swedish University of Agricultural Sciences

**Uppsala 2005**

Report - Master of Science Programme in Veterinary Medicine  
for International Students  
Faculty of Veterinary Medicine and Animal Science  
Swedish University of Agricultural Sciences (SLU)  
Report no. 54  
ISSN 1403-2201

# **Sperm morphology in progeny-tested Swedish AI dairy bull sires**

**Anas Al - Makhzoomi**

Division of Comparative Reproduction, Obstetrics and Udder Health  
Department of Clinical Sciences  
Faculty of Veterinary Medicine and Animal Science

**Swedish University of Agricultural Sciences  
Uppsala 2005**

The present thesis is a partial fulfilment of the requirements for a Master of Science Degree in Veterinary Medicine for International Students (MSc), at the Swedish University of Agricultural Sciences (SLU), in the field of **Animal Reproduction**

Anas Makhzoomi  
Division of Comparative Reproduction, Obstetrics and Udder health  
Department of Clinical Sciences  
Faculty of Veterinary Medicine and Animal Science  
Swedish University of Agricultural Sciences (SLU)  
P.O. Box 7054, SE- 75007 Uppsala, Sweden  
Print: SLU Service/Repro, Uppsala 2005



*To my parents (Associate Professor Khalaf Al-makhzoomi and Mona Sulieman), brothers (Alla and Mohammad), sisters (Assistant Professor Ibtihal, Batool and Afya) and my sweet lovely wife (Faten Al-Yabroudi) ...*

## ABSTRACT

**Makhzoomi, A. 2005.** Sperm morphology in progeny-tested Swedish AI dairy bull sires. Master's thesis. ISSN 1403-2201, Report no. 54.

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. If sperm morphology deteriorates with age, it becomes 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, an 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 bull within breed age affected some semen (ejaculate volume) and most sperm morphology variables, while age significantly affected only few sperm morphology variables (nuclear pouches and sperm head contour). Estimated breeding values for male fertility output differed significantly among bulls and, when all ejaculates were accounted for, the percentage of morphologically normal spermatozoa was positively related to fertility. Few individual sperm abnormalities in all ejaculates correlated significantly to fertility, as pear-shaped sperm heads. When separated by breed, these correlations were restricted, in SRB bulls, to the normal spermatozoa in wet smears, or lost in SLB bulls, probably owing to the reduced sire material examined. In conclusion, the presence of some 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.

**Author's address:** Anas Makhzoomi, Division of Comparative, Division of Comparative Reproduction, Obstetrics and Udder Health, Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), PO Box 7054, SE-750 07, Uppsala, Sweden.

# Contents

## **Background, 9**

Artificial insemination in dairy cattle, 9

*Historical summary, 9*

*Testing of bull sires, 11*

Assessment of semen quality and its relation to fertility, 12

## **Introduction to the research report, 15**

**Aim of the study, 16**

## **Research report, 17**

*Sperm morphology and fertility of progeny-tested AI Swedish dairy bulls, by Makhzoomi A, Lundeheim N, Håård M & Rodríguez-Martínez H*

**General conclusions, 35**

**Future prospects, 36**

**General references, 37**

**Acknowledgements, 40**

# Background

## Artificial insemination in dairy cattle

Artificial Insemination (AI), the manual deposition of spermatozoa in the reproductive tract of a sexually receptive female by the use of artificial means, is considered the first reproductive biotechnology. Created with the major intention of controlling the dissemination of venereal diseases, AI still remains as the most important technology for safe gene dispersion in livestock breeding (Vishwanath, 2003). The AI-technology has also provided impetus for developing other assisted reproduction techniques such as cryopreservation of semen, sperm sexing, as well as alternative methods for semen deposition, oestrous cycle regulation and control. Artificial insemination has also maintained association with embryo transfer (Foote, 2002).

Artificial insemination has facilitated the choice of using the best possible bulls of proven quality in improving the genetic make up of the cattle population thus conveying to the primary goal for breeding; to increase the productivity and the profitability of a particular commercial herd by increasing the number of offspring produced by selected genetically superior bulls. The use of AI made also possible to measure the performance of large numbers of progenies born after AI of many females using sperm doses from a single bull, thus allowing the accurate selection of bulls with desirable characters.

### *Historical summary*

Artificial Insemination has been anecdotically described as firstly performed as early as the 14<sup>th</sup> century by an Arabic horse breeder, who transferred the contents of an ejaculate from the vagina of a recently served mare into the vagina of another mare by means of a sponge. The first documented AI is not that old, performed by the Italian abbot and researcher Spallanzani who, in 1784, artificially inseminated a female dog which whelped three pups 62 days later (Foote, 2002). Spallanzani filtered the semen later on and found that only the remained sperm fraction of the ejaculate, and not the filtrate, could cause the fertilization (Foote, 1999).

In 1899, Ivanov developed the first organized AI research studies in Russia, developing semen extenders and training technicians for the selection of superior stallions and the development of AI to multiply their breeding outcome (Foote, 1999). By using AI, Ivanov and his co-workers hoped to restrict the spread of venereal diseases of cattle, the most relevant rationale for the use of this technology, in veterinary terms. In 1914, Amantea developed the first artificial vagina for the use in dogs (Perry, 1968). This work served as a model for the Russian development of artificial vaginas for bulls (Foote, 2002). Later, in 1931, the state farms started to use AI, thus exponentially increasing its use in livestock breeding (Rodin and Lipatov, 1936). In Japan, AI began as a new technique in 1912 after the successful studies of Ishikawa with Ivanov, contributing to the spreading of the technology (Nishikawa, 1962). In 1937, cattle AI became a

practical commercial scheme when Sorensen, a Danish Veterinarian who was familiar with the Russian AI-programmes, developed the first cooperative dairy AI organization in Denmark (Sorensen, 1938). His successful achievements were important stimuli for the development of AI in dairy cattle in the USA (1938) and other western countries (Perry 1968; Foote, 2002). Meanwhile, the Danish Veterinarians established the procedure of recto-vaginal fixation of the cervix; a praxis still used today for intrauterine AI, that increased fertility with a reduced sperm number and made -eventually- possible the use of frozen-thawed semen.

The development of an egg yolk-phosphate semen extender for liquid semen stored for some days was the first major improvement enhancing AI (Phillips and Lardy, 1940), being followed by Salisbury et al. (1941) who buffered the extender with sodium citrate (Foote, 2002). A major breakthrough was the discovery (by fortunate chance) that chicken spermatozoa could be effectively frozen and thawed by the addition of glycerol as cryoprotectant (Polge et al., 1949). The addition of glycerol to the original yolk-citrate extender, increased fertility by 15% units, since spermatozoa could then be protected from cold shock (Foote and Bratton, 1949). Another major breakthrough was the control of venereal diseases by the routinary addition of antibiotics to the extender (Almquist et al., 1949; Foote and Bratton, 1950).

In the early 1950s, powerful tools for progeny testing were developed by Henderson and Robertson (cf Van Vleck, 1981). Few years later and for the first time, frozen semen was routinely used by a Canadian organization (Waterloo), followed by the American Breeders Service in 1957, which improved the liquid nitrogen tanks and service for frozen semen. In 1963, the Tris-buffered egg yolk – glycerol extender was developed which provided excellent protection for the spermatozoa either frozen or unfrozen (Davis et al., 1963; Foote, 1998). This modified extender became the most commonly used medium for cryopreservation of the semen of most species (Iritani, 1980). In 1964, Cassou modified the system originally developed by Sorensen (1940), with a method used for sealing plastic straws and a gun for insemination (Pickett and Berndtson, 1974). During the past 40 years, the development and application of cattle AI with preserved semen have been growing exponentially and on a global scale, with more than 100 million of first AIs in the world using frozen semen (Thibier and Wagner, 2000).

The initiation of AI in Denmark in the early 1930s by Sorensen, encouraged Swedish researchers to focus on the remarkable value of such technique in eradicating venereal diseases and contributing to cattle health management. By 1935, two members from the Animal Breeding Institute at Wiad; Kåre Backström; a biologist and Gert Bonnier; a professor in animal breeding, made study tours to Denmark and Russia. Their enthusiasm for the potential value of AI along with the help of specialist Veterinarians, such as Allan Bane; at the Institute of Animal Breeding, resulted in the establishment of training programs on this new field in 1942. In the year after, the first farmer-driven AI associations were founded in Scania (Swensson, 1993). Soon, knowledge of this new technology rapidly expanded into cattle breeding through agricultural organizations, agricultural publications and local press. The first National Organization of AI Co-operatives

(RSS) was founded in 1944, helping the AI societies in designing breeding plans, financial matters, equipment, terms of employment for the staff and training of technicians. In 1946, Professor Nils Lagerlöf established training programs in AI, expanding the application of this technique to include not only veterinarians but also field-technicians. In 1947, nineteen A.I centers were established with 89,728 AIs being applied (Swensson, 1993). By 1950, testing and development of different extenders in order to prolong semen durability was soon followed by implantation of deep-freezing of semen, first in glass ampoules, then in medium-payettes and mini-Payettes (0.25 mL) the latter dominating since 1971. Until the year 2000, 90% of the dairy cows and heifers were solely bred by AI, a figure that is still maintained at present, among the approx 300,000 dairy females being artificially inseminated in Sweden.

### *Testing of bull sires*

In Sweden, a group of approximately 200 young bulls (2/3 SRB) are taken into Svensk Avel ek. för.; a nationally dominating AI enterprise own by farmer organisations, after being raised in a rearing station until the age of approx 11-12 months where they follow a testing based on conformation and growth rate. These young bulls were recruited as calves (among a total number of 400-500 calves), based on the genetic value of their ancestors (particularly conformation, production, growth and health), tested for specific diseases, and clinically surveyed. Semen is collected from these young bulls and, following a immediate control of semen quality mainly based on volume, concentration and sperm motility, the semen is challenged for its ability to be cryopreserved using a single set up for handling and freezing/thawing procedures. Those bulls whose semen lies within normal parameters and sustains these procedures for freezing and thawing is then used to inseminate cows and heifers in herds all over the country, based on a scheme where members of the farmers organisation comply with by allowing AI of a certain percentage of their herd with semen from these unproven young bulls. The results of the AIs are collected as non-return-rates (NRR, e. g. the percentage of cows not returning to oestrus at some, most often 56 day, interval post-AI). Since such estimates of fertility are under the influence of several factors (season, inseminator, area, category of female etc) that can overestimate its outcome, NRRs are statistically corrected for the influence of these factors and called adjusted values or estimated breeding values. In average, the AIs by semen from a given sire result in a daughter group of 150 females per tested bull. This female progeny, of those sires that attain an acceptable fertility level, is thereafter monitored during their first lactation and the resulting analyses provide production data that is accumulated during the waiting period of progeny testing. The accumulated data is used to select genetically superior bulls for return to AI service and be maintained for regular production of commercial semen and to become parents of the next generation of females. A small proportion of these proven bulls are also used to sire the next bulls to be progeny-tested. After progeny testing, only a small percentage (approx. 5%, with variation between years) of the progeny tested bulls are selected as proven bull sires.

## **Assessment of semen quality and its relation to fertility**

At Svensk Avel, a young unproven bull should produce semen with a total sperm number of  $\geq 2.5 \times 10^9$  per ejaculate and have  $\geq 65\%$  of spermatozoa depicting progressive rectilinear motility to be considered for freezing. If screened further, such semen should also have  $>90\%$  of the total number of spermatozoa depicting morphologically normal sperm head shape,  $<5\%$  of morphologically immature spermatozoa (e.g. having proximal cytoplasmic droplets) and  $<10\%$  of sperm tail abnormalities. Furthermore, it should contain neither inflammatory cells nor rich amounts of other foreign cells. Within such a range the semen would be declared as having a normal semen picture as the expression of normal testicular production, normal epididymal sperm maturation and normal function of the accessory sexual glands. The sire should, if found able to mount and showing a normal libido, be apt for reproduction and potentially fertile.

Decades ago, AI doses contained 30 million spermatozoa. Figures of 7.5-10 million spermatozoa/dose are much more common nowadays (Den Daas et al., 1998; Håård and Håård, 2000). Such decrease in the total number of spermatozoa/dose are a consequence of gained knowledge in the methods to handle and freeze/thaw semen as well the fact that spermatozoa from some bulls can still maintain acceptable fertility post-AI at high-extension rates, while others can not. This means that for each bull, there is a certain number of spermatozoa that will maintain viability at thawing and, consequently, present a minimal limit per dose, warranting the expression of a certain level of individual fertility. For proven bulls, the range of viable spermatozoa per AI-dose calculated as needed to obtain 95% of maximal conception varies between 1 and 11 million spermatozoa (Den Daas et al., 1998).

When frozen-thawed semen is used for AI, the apparent fertility of the majority of sires follows a dose response curve in relation to the total number of viable, normal spermatozoa. Such a curve reaches an individual plateau of maximal fertility (usually below 100%) after showing a marked increase that also follows a different slope for each individual animal (Pace et al., 1981; Umland, 1984). There is a statistically significant relationship between the total number of viable spermatozoa inseminated and the fertility post-AI, following linearly the fertility level for each sire (Den Daas et al., 1998, Schwartz et al., 1981; Shannon and Vishwanath, 1995). This means that the insemination of additional spermatozoa from a sub-fertile bull would not necessarily result in better fertility than the one originally shown by the sire (Pace et al., 1981; Den Daas et al., 1998). For this reason, AI semen producers conduct nowadays dose-response curves for sperm numbers in the AI-dose for each sire, in order to determine the doses that warrant their maximal fertility (usually between 5 and 20 million spermatozoa).

The above results coincide with the concept presented by Saacke et al. (1998); sperm characteristics whose deficiencies are reflected in reduced gestation rates can be categorized as compensable or not compensable. Those that are compensable are relevant for sperm transport, including the interaction with the female genital tract and the vestments of the oocyte (includes sperm viability, motility and some morphological features), while those named uncompensable are

relevant to maintain the processes of fertilization and early embryo development (morphology, genomic integrity and intactness of the centriole, for instance). Increasing the number of spermatozoa in the AI-dose can only compensate for the first category. However, when maximal fertility is reached (see below), increasing the sperm number has no effect, and the differences in fertility among males are then considered as being determined by the non-compensable attributes of the spermatozoa (Saacke et al., 2000).

Sperm motility is the parameter most frequently used to measure sperm viability in the ejaculate and during the process of cryopreservation, and although it has been related to fertility, the correlations varied largely among studies, from highly related to poor correlations (Kjaestad et al., 1993; Stålhammar et al., 1994; Bailey et al., 1994). Using computerized equipments (CASA), the analyses of standardized semen samples from bulls have shown that certain patterns of post-thaw motility, such as linearity, correlate significantly with field fertility ( $r^2 = 0.45-0.63$ , Zhang et al., 1998; Januskauskas et al., 2001; 2003). Even stronger correlations could be found when various motility patterns were combined statistically with other parameters of sperm function ( $r^2 = 0.68-0.98$ , Farrell et al., 1998; Januskauskas et al., 2001) in AI-bulls. Motility depends on the ability of spermatozoa to produce and consume ATP. The ATP contents can be measured by luminometry (Söderquist, 1991) but neither the ATP contents nor the quantitation of viable sperm numbers, as indirectly determined by ATP, seem to correlate with fertility post-AI (Januskauskas et al., 1996). Spermatozoa can not live nor interact with the female tract or the oocyte without an intact plasma membrane. Therefore, evaluation of the sperm plasma membrane integrity has been one of the parameters most studied lately, either morphologically (in stained or wet smears, using light microscopy), using specific fluorescent probes (and analyses of microscopy or flow cytometry) or incubations in hypo-osmotic solutions (for review, see Rodriguez-Martinez et al., 1998). Analyses of membrane integrity that screen large numbers of spermatozoa yield significant correlations to fertility (Ericsson et al., 1993; Januskauskas et al., 2001). The intactness of the acrosome, either assessed morphologically or by fluorescent probes has been statistically related to the fertility of frozen-thawed semen (Graham et al., 1990; Januskauskas et al., 2000a, b).

The evaluation of the degree of denaturalization of the DNA using flow cytometry (the so-called "Sperm Chromatin Structure Assay", SCSA, [Evenson et al., 1980; 1982]), a method that measures the degree of increment of structural heterogeneity of the sperm chromatin, has been associated to fertility (Ballachey et al, 1998; Evenson and Jost 2000; Januskauskas et al., 2001; 2003) and appears as a valuable complement to the microscopic assessment of sperm morphology (Evenson, 1999) to measure disturbances of the spermatogenesis that could cause morphological deviations in the morphology of the sperm head.

The evaluation of sperm morphology is an important component of the spermogramme and therefore of the andrological evaluation. Sperm morphology indicates normality or eventual deviations of the spermatogenesis and sperm maturation in the epididymis and its results, correctly assessed, are used to cull males with semen of low quality for AI when these suggest major genital

pathologies (Lagerlöf, 1934; Söderquist, 1991). Unfortunately, when the morphological parameters are within acceptable limits, the morphology of the sample does not, *per se*, provides enough information to judge the expected level of fertility of the semen post-AI. This is independent of a subjective assessment (Zhang et al 1998) or the use of computer-based equipment (ASMA, Gravance et al., 1999; Boersma et al., 2000), since, in many cases, a high sperm number in the dose is able to compensate, at least partially, a high percentage of morphological abnormalities (Saacke et al., 1998). This is especially clear for the bull, where the degree of selection for sperm quality (including morphology) has determined that the incidence of sperm abnormalities in bulls of similar age has decreased substantially within 30 years (compare Hultnäs, 1959 with Söderquist et al., 1991). Bull spermatozoa with certain sperm head morphological deviations (such as those with nuclear pouches in heads with normal contour) do sometimes reach the oocyte (Saacke et al 1998), but not in all cases (Thundathil et al., 1998). In any case, they are related to lower fertilization rates and embryo development (Thundathil et al., 1998). Other defects, such as the “knobbed acrosome”, prevents the union of sperm-ZP (Thundathil et al., 2000), while others (pyriform sperm heads) have a reduced capacity to bind and penetrate the ZP *in vitro*, and the resulting zygotes cleave abnormally (Thundathil et al., 1999). There is, however, a relationship between sperm morphometry (bull semen) and the normal structure of the chromatin (the latter in direct relation to fertility; see above, Sailer et al., 1996). Recently, the percentage of morphologically normal spermatozoa has been identified as a parameter statistically related ( $r= 0.54$ ) to field fertility (corrected conception rates, Phillips et al., 2000).

Sperm quality has been found as differing with the age of the sires (Almquist and Amann, 1976; Saeed et al., 1989). 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 has been studied and set for this age-interval (Söderquist et al., 1991). Most often, this setting is also used for bulls of an older age, including progeny-tested bulls. Recently, Hallap et al. (2004) has studied sperm morphology post-thaw in bulls when young (11-13 months of age) as well as while these were awaiting progeny testing (4 years of age), finding that while sperm motility and membrane integrity improved when sires were older, sperm morphology did not reveal any significant changes with age. Since this study was done on frozen-thawed samples, it remains to be determined whether age influences sperm morphology in ejaculated, not-processed semen of progeny-tested bulls.

## **Introduction to the research report**

Dairy bulls providing semen for AI are tested for fertility at an early age (usually 11-13 months of age). Sperm morphology thresholds for normality have been studied and set for this age-interval (Söderquist et al., 1991), a setting that is routinely also used for bulls of an older age, including progeny-tested bulls. Since some data indicate that sperm quality differs with the age of the sires, it may be assumed that such changes could indeed affect the morphology of the spermatozoa and even the fertility of the sires. Such assumption is based on the fact that the genital normality of these bulls could have changed over time and this would affect the quality of the semen they produce. Therefore, there a logical need to determine which levels of sperm abnormalities are present in progeny-tested bulls, with the aim of setting proper thresholds and also to find out if these levels of abnormalities could be related to the fertility of the proven sires.

## **Aim of the study**

The overall aim of the study was to increase our knowledge about the relationship between the overall level of sperm abnormalities in the semen of progeny-tested dairy 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. As well, it aimed to determine the variation in sperm morphology within bull and the influence of age and breed on the outcomes. The hypothesis tested was that fertility after AI is dependent on the morphology of the spermatozoa to be cryopreserved and used for AI.

# Research Report

## Sperm morphology and fertility of progeny-tested AI Swedish dairy bulls

Makhzoomi, A.<sup>1</sup>, Lundeheim, N.<sup>2</sup>, Håård, M.<sup>3</sup> & Rodríguez-Martínez, H.<sup>1</sup>

*<sup>1</sup>Division of Comparative Reproduction, Obstetrics and Udder Health, Department of Clinical Sciences, <sup>2</sup>Department of Animal Breeding and Genetics, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), Ullsvägen 14 C, Clinical Centre, PO Box 7054 Ultuna, SE-750 07 Uppsala, <sup>3</sup>Svensk Avel ek. för., Örnsro, 532 94 Skara, Sweden*

### 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. If sperm morphology deteriorates with age, it becomes 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, an 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 bull within breed age affected some semen (ejaculate volume) and most sperm morphology variables, while age significantly affected only few sperm morphology variables (nuclear pouches and sperm head contour). Estimated breeding values for male fertility output differed significantly among bulls and, when all ejaculates were accounted for, the percentage of morphologically normal spermatozoa was positively related to fertility. Few individual sperm abnormalities in all ejaculates correlated significantly to fertility, as pear-shaped sperm heads. When separated by breed, these correlations were restricted, in SRB bulls, to the normal spermatozoa in wet smears, or lost in SLB bulls, probably owing to the reduced sire material examined. In conclusion, the presence of some 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.

**Short title:** Sperm morphology in progeny tested AI sires

**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 & 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. The 56d-NRR 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 rates are under the influence of several factors that can overestimate the outcome. Examples of these factors are the reliability of the oestrus 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 to 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 -rather than often- the semen of the sires is assessed for sperm morphology, an evaluation being done when suspicion 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, epididymides or accessory sexual glands (Lagerlöf, 1934), but also associated with a decreased fertility of the semen (Saacke, 1970). 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 (rev by Rodriguez-Martinez, 2003).

Sperm quality differs with the age of the sires (Almquist and Amann, 1976; Saeed et al., 1989). 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). Often, these thresholds are also used for bulls of an older age, including proven bulls. Recently, Hallap et al. (2004) has 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 this study was done on frozen-thawed samples, it remains to be determined whether age influences sperm morphology in ejaculated, not-processed semen of progeny-tested bulls. Moreover, 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 retrospective study was to examine relationships 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 and breed as well as the variation between- and within-bull for presence and level of sperm abnormalities.

## **Materials and Methods**

### **Source of semen**

Semen collected from eight 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 (Örmsro, 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 for 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-dried, 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 cytoplasmic 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. Relative proportions of morphologically normal spermatozoa were estimated as mean percentage of the remaining spermatozoa without defects counted in wet smears and the remaining spermatozoa without head shape defects counted in stained smears. A thick, dried smear stained with haematoxylin-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) as well as inflammatory cells (leukocytes, lymphocytes, monocytes/macrophages).

The relative presence of each foreign cell type was classed as 0= absent, 1= scarce, 2= moderate, 3= rich to 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) (e.g. 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 using 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  $\geq 80$  months of age) and bull nested within breed (4 SLB; 8 SRB). Residuals were calculated, and these residuals were correlated (using Spearman correlation) within breed. Differences were considered significant when  $p < 0.05$ .

**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 the evaluated sires (SRB: Swedish red and white breed; SLB: Swedish Holstein breed; Mean  $\pm$  SD).

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

## Results

The progeny-tested sires were examined for sperm morphology when they averaged  $77.0 \pm 7.0$  months of age. The characteristics of the collected ejaculates are presented in **Table 1**. Semen volume averaged  $(6.0 \pm 1.5$  mL, with an overall mean total sperm number of  $8.4 \pm 3.4$  billion spermatozoa. Initial sperm motility averaged  $72.0 \pm 2.4$  % while the post-thaw sperm motility of the frozen batches averaged  $52.6 \pm 2.4$  %. Significant differences were detected for the variables 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 either 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 relative overall frequency of spermatozoa depicting normal morphology varied significantly among sires ( $P < 0.01$ ) and between breeds ( $P < 0.001$ ), but was not significantly affected by age ( $P = 0.0825$ ). Overall, the percentage of total sperm head abnormalities showed a mean of  $15.0 \pm 11.0$ %, with 6/12 sires having more than 10% of sperm head abnormalities (highest mean was  $36.7 \pm 7.9$ %). However, the percentages of individual sperm abnormalities varied largely among bulls ( $P < 0.001$ ) and ejaculates ( $P < 0.05$ ).

The highest mean value for individual sperm defects within bull was recorded for pear-shaped sperm heads (20.7 %, bull H), two bulls having significantly higher values than the rest of the sires ( $P < 0.05$ ) (see **Table 2**). Differences were found for most sperm abnormalities, but with the exception of pear-shaped or narrow at the base anomalies, 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  $\pm$  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 were present among bulls for corrected fertility values ( $P < 0.001$ ).



**Table 2:** Percentages of sperm abnormalities in semen from proven sires (A-L) of the Swedish red and white (SRB) or Swedish Holstein (SLB) breed (mean  $\pm$  SD).

Sperm abnormality		Bulls												
		SRB								SLB				All bulls
		A	B	C	D	E	F	G	H	I	J	K	L	
Head	Pear shaped	7.6 $\pm$ 2.7	6.5 $\pm$ 2.5	6.1 $\pm$ 1.6	2.9 $\pm$ 1.1	1.2 $\pm$ 0.6	2.5 $\pm$ 1.9	2.3 $\pm$ 1.5	20.7 $\pm$ 5.5	4.1 $\pm$ 1.8	2.1 $\pm$ 1.2	10.4 $\pm$ 0.8	1.8 $\pm$ 0.8	5.7 $\pm$ 5.5
	Narrow at base	6.7 $\pm$ 2.5	3.3 $\pm$ 1.4	1.8 $\pm$ 0.9	3.0 $\pm$ 1.0	1.8 $\pm$ 0.8	2.1 $\pm$ 1.6	0.6 $\pm$ 0.5	8.1 $\pm$ 3.1	8.0 $\pm$ 1.6	1.9 $\pm$ 0.6	8.7 $\pm$ 0.1	1.9 $\pm$ 0.9	4.0 $\pm$ 3.0
	Narrow	0.8 $\pm$ 0.5	0.2 $\pm$ 0.2	0.1 $\pm$ 0.2	0.4 $\pm$ 0.4	0.6 $\pm$ 0.5	0.5 $\pm$ 0.7	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2	0.4 $\pm$ 0.3	1.0 $\pm$ 0.5	0.1 $\pm$ 0.1	0.5 $\pm$ 0.6	0.4 $\pm$ 0.2
	Abnormal contour	0.6 $\pm$ 0.5	0.4 $\pm$ 0.2	0.4 $\pm$ 0.4	0.2 $\pm$ 0.3	0.3 $\pm$ 0.3	0.3 $\pm$ 0.2	0.4 $\pm$ 0.3	0.3 $\pm$ 0.3	0.2 $\pm$ 0.2	0.4 $\pm$ 0.5	0.1 $\pm$ 0.1	0.3 $\pm$ 0.4	0.3 $\pm$ 0.1
	Variable size	1.1 $\pm$ 0.6	1.9 $\pm$ 0.7	2.6 $\pm$ 1.3	0.7 $\pm$ 0.8	1.3 $\pm$ 0.7	1.2 $\pm$ 0.4	1.9 $\pm$ 0.7	1.6 $\pm$ 0.8	0.6 $\pm$ 0.3	1.4 $\pm$ 0.8	1.4 $\pm$ 0.9	0.9 $\pm$ 0.8	1.4 $\pm$ 0.5
	Loose abnormal	4.7 $\pm$ 3.2	0.6 $\pm$ 0.5	0.7 $\pm$ 0.7	0.2 $\pm$ 0.2	0.2 $\pm$ 0.3	0.4 $\pm$ 0.4	0.2 $\pm$ 0.2	4.8 $\pm$ 1.9	0.1 $\pm$ 0.2	0.4 $\pm$ 0.3	1.3 $\pm$ 0.8	0.2 $\pm$ 0.2	1.3 $\pm$ 1.9
	Underdeveloped	1.1 $\pm$ 0.9	0.9 $\pm$ 0.4	1.7 $\pm$ 1.1	0.4 $\pm$ 0.4	0.2 $\pm$ 0.2	1.0 $\pm$ 0.4	1.1 $\pm$ 0.4	0.9 $\pm$ 0.4	0.4 $\pm$ 0.4	0.5 $\pm$ 0.7	0.6 $\pm$ 0.5	0.4 $\pm$ 0.3	0.7 $\pm$ 0.4
	Nuclear pouches	0.4 $\pm$ 0.5	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.9 $\pm$ 0.9	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0	0.2 $\pm$ 0.2
	Acros. defect	0.2 $\pm$ 0.3	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.2 $\pm$ 0.4	0.4 $\pm$ 0.4	0.1 $\pm$ 0.1	0.5 $\pm$ 0.7	0.2 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.0 $\pm$ 0.0	0.2 $\pm$ 0.1
	Acros. abnormal.	2.6 $\pm$ 2.2	1.9 $\pm$ 1.1	2.0 $\pm$ 1.4	1.4 $\pm$ 0.9	1.3 $\pm$ 0.9	2.1 $\pm$ 0.7	2.4 $\pm$ 1.7	6.0 $\pm$ 2.5	1.6 $\pm$ 1.9	2.4 $\pm$ 1.7	1.9 $\pm$ 0.4	3.2 $\pm$ 2.1	2.3 $\pm$ 1.2
Abaxial	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.4 $\pm$ 0.3	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1	
Neck	Loose heads	8.3 $\pm$ 5.8	1.2 $\pm$ 1.0	1.2 $\pm$ 1.0	0.8 $\pm$ 0.8	1.6 $\pm$ 1.2	2.1 $\pm$ 1.9	0.8 $\pm$ 0.5	5.8 $\pm$ 2.4	0.4 $\pm$ 0.7	1.2 $\pm$ 1.4	2.7 $\pm$ 1.0	1.3 $\pm$ 1.0	2.4 $\pm$ 2.8
Cyt. drops	Proximal	1.7 $\pm$ 0.5	1.5 $\pm$ 0.9	1.0 $\pm$ 0.9	2.2 $\pm$ 0.9	0.6 $\pm$ 0.6	2.4 $\pm$ 0.5	2.2 $\pm$ 0.9	8.8 $\pm$ 4.9	0.3 $\pm$ 0.4	0.9 $\pm$ 0.8	0.6 $\pm$ 0.4	5.3 $\pm$ 6.3	2.3 $\pm$ 2.4
	Distal	17.5 $\pm$ 11.5	4.0 $\pm$ 3.4	9.7 $\pm$ 7.3	2.6 $\pm$ 1.6	5.9 $\pm$ 6.8	4.3 $\pm$ 6.2	6.9 $\pm$ 6.9	10.4 $\pm$ 7.0	0.8 $\pm$ 0.6	5.1 $\pm$ 4.0	2.0 $\pm$ 3.1	13.0 $\pm$ 14.0	6.6 $\pm$ 4.5
Mid-piece	Abn. midpiece	0.8 $\pm$ 0.9	0.6 $\pm$ 0.5	0.9 $\pm$ 0.7	0.4 $\pm$ 0.4	0.8 $\pm$ 0.9	2.0 $\pm$ 0.9	0.7 $\pm$ 0.7	1.1 $\pm$ 0.8	0.9 $\pm$ 0.8	1.2 $\pm$ 0.9	1.0 $\pm$ 0.8	0.9 $\pm$ 0.4	0.9 $\pm$ 0.4
Tail	Simple bent	2.0 $\pm$ 2.4	2.8 $\pm$ 3.1	2.3 $\pm$ 1.5	0.3 $\pm$ 0.3	0.9 $\pm$ 0.7	0.5 $\pm$ 0.5	0.3 $\pm$ 0.2	5.5 $\pm$ 3.3	1.0 $\pm$ 0.8	1.2 $\pm$ 0.6	2.3 $\pm$ 0.8	2.0 $\pm$ 1.7	1.7 $\pm$ 1.4
	Under the head	0.9 $\pm$ 0.9	0.9 $\pm$ 0.7	1.3 $\pm$ 0.9	0.2 $\pm$ 0.3	0.1 $\pm$ 0.2	0.4 $\pm$ 0.2	0.3 $\pm$ 0.3	1.6 $\pm$ 1.2	0.3 $\pm$ 0.4	0.6 $\pm$ 0.5	0.3 $\pm$ 0.3	0.8 $\pm$ 0.4	0.6 $\pm$ 0.4
	Double-folded	0.4 $\pm$ 0.5	0.4 $\pm$ 0.4	0.8 $\pm$ 0.5	0.1 $\pm$ 0.2	0.9 $\pm$ 0.4	0.4 $\pm$ 0.4	0.3 $\pm$ 0.5	0.7 $\pm$ 0.7	0.1 $\pm$ 0.2	1.4 $\pm$ 1.4	0.7 $\pm$ 0.8	0.5 $\pm$ 0.8	0.6 $\pm$ 0.3



**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  $\pm$  SD.

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

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 ( $P < 0.05$ - $0.001$ ). 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 ( $r = 0.22$ ,  $P < 0.05$ ), the relations for individual morphological defects and fertility were few. Considering all ejaculates, pear-shaped sperm heads were negatively related to fertility ( $r = 0.80$ ,  $P < 0.01$ ). Differences were present between breeds. While for SRB bulls the percentage of normal spermatozoa correlated positively with fertility ( $r = 0.25$ ,  $P < 0.05$ ), those of loose heads and proximal droplets were negatively related to fertility ( $r = -0.39$ ,  $P < 0.01$  and  $r = -0.29$ ,  $P < 0.05$ , 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, albeit few, sperm abnormalities were negatively correlated to fertility.

Significant differences were found between the two breeds with regard to the corrected fertility and morphological defects. No significance 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 difficult to compare the outcomes of these studies.

Sperm morphology varied significantly between breeds as well as between sires within breed. Age influenced few variables, such as total sperm numbers per ejaculate and few individual sperm abnormalities (nuclear pouches and abnormal sperm head contour). The latter 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 to 26 months, or the findings from Hallap et al (2004) that registered no significant difference in sperm morphology in bull sires when aged 1 or 4 years. Individual differences and differences in age range can be behind the differences encountered. Moreover, since 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 peak maximal sperm production and when an eventual ageing effect starts.

Fertile bulls have more viable spermatozoa and a consistently lower incidence of morphologically abnormal sperm than non-fertile or sub-fertile bulls (Lagerlöf, 1934; Barth and Oko, 1984; Salisbury *et al.*, 1978; Saacke, 1982). Thus, bulls that fail in a breeding soundness evaluation generally have more sperm abnormalities than those who pass (Spitez *et al.*, 1988). Evidence of a relationship between sperm morphological characteristics of spermatozoa and individual differences in fertility was first presented by Williams in 1926 and Lagerlöf in 1934. Sperm abnormalities have traditionally been classified by sperm domain (head, tail, mid-piece, cytoplasmic droplets), relative importance (major or minor defects), or site of origin (primary: testis; secondary: epididymis; tertiary: accessory glands/post ejaculation). In 1934, Lagerlöf established a classification system that illustrated the relationship between different sperm abnormalities of fresh semen and fertility

in bulls used in natural service, his observations showing that the abnormalities in the fresh semen were correlated to the histopathological alterations found in the testes of low fertility bulls. This was the basis for the use of examination of sperm morphology as a test of semen quality in young AI bulls (Söderquist, 1991). In 1974, Morstin and Courot showed that ultrastructural changes of testicular origin in the nucleus, acrosome and mitochondria of spermatozoa occurred more frequently in low fertility AI bulls. However, studies by Gustafsson (1966) have shown that some sperm abnormalities, such as non-specific sperm tail abnormalities, do not originate in the testis but during epididymal transit.

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). 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.

As stated before, sperm abnormalities can be classified for their presence and their relation to pathologies in the testis (Lagerlöf, 1934). Sperm abnormalities can also be grouped for their significance as compensable or uncompensable (rev by Saacke *et al.*, 1998). Inability to undergo transport to the uterine tube or to penetrate the zona pellucida can be compensated by increasing the number of spermatozoa in the sperm dose. Those sperm abnormalities considered uncompensable are, however, present in spermatozoa which are perhaps capable of penetrating the zona pellucida of the oocyte, but which fail to cause cleavage or result in non-viable embryos. Such defects can not be compensated by simply increasing the number of spermatozoa in the sperm dose used for AI (Saacke, 1982; Barth, 1997). Abnormalities of the sperm head or the presence of proximal cytoplasmic droplets reflect defects of nuclear chromatin condensation respectively immaturity of the spermatozoa and are likely to be uncompensable (Parkinson, 2004). Studies by Saacke (1970) have shown that high percentages of such abnormal spermatozoa in a semen sample are correlated with a lower fertility of the bull, a relationship confirmed in the present study. Sperm abnormalities can also be classed according to their effect upon fertility into major and minor defects (Blom, 1983). Major defects include most abnormalities of the head and mid-piece, proximal cytoplasmic droplets and single abnormalities that are present at high frequency. Minor defects include looped tails, detached sperm heads and distal cytoplasmic droplets. In the present study, sperm defects were not classed according to any of these classifications but listed when present in each spermatozoon counted. Abnormalities that compromise the sperm head shape, and that most likely occur during spermiogenesis, are responsible for a lowered fertility when semen is used for AI. Being such abnormalities not compensable, freezing batches of bulls with more than 10% of abnormal sperm head morphology

in the ejaculate would risk a lower fertility even with a relatively higher sperm number in the straw.

Wilmington (1981) and Söderquist et al. (1991) have stated that a significant impairment of fertility in bulls can be associated with the presence of sperm head abnormalities in their semen. Such abnormalities are very important since they adversely affect the binding and passage through the zona pellucida (ZP). For instance, using IVF as a test environment, Thundathil et al. (2000) have found that no spermatozoa with the knobbed acrosome defect could penetrate the ZP and those embryos which were fertilized by 'normal' spermatozoa from affected bulls had a slower rate of cleavage than those from control bulls. Using the same methodological approach, spermatozoa with a misshapen pyriform head (pear-shaped head), a deformity appearing in testicular hypoplasia and as an acquired defect during testicular degeneration, impaired both fertilization rate and subsequent embryonic development (Thundathil et al., 1999).

The degree of deformity of the sperm head has been related to decreased fertility (Petac and Kosec, 1989; Nöthling and Arndt, 1995), and suggested to be as a consequence of a reduction of its total surface area (Barth et al., 1992). Some bulls normally produce spermatozoa that are relatively pyriform in shape and, as a consequence, suggested to be relatively sub-fertile (Parkinson, 2004). If a defective chromatin condensation occurs during spermiogenesis and leads to these morphological deviations, a defective decondensation during pronuclear formation can very well be behind the reported infertility caused by high levels of pear-shaped sperm heads. However, conclusive studies are yet to be performed in order to determine the way pear-shaped sperm heads compromise fertility. Noteworthy, the highest mean value for individual sperm defects/bull was, in the present study, recorded for pear-shaped sperm heads (20.7 %). Considering pyriform-shaped sperm heads appear as importantly related to fertility decrease, the presence of this particular abnormality should be carefully monitored.

Few other sperm abnormalities were above what is expected as within acceptable limits such as presence of proximal cytoplasmic droplets or distal cytoplasmic droplets. The significance of these defects is different when related to fertility. Distal cytoplasmic droplets are not generally regarded as serious abnormalities although being at high percentages (Barth, 1997). Substantial impairment of fertility results, however, when proximal droplets are present (Linford et al., 1976; Blom, 1977; Söderquist et al., 1991; Saacke et al., 1995). Furthermore, when sperm with proximal droplets have been used in IVF, cleavage rates of embryos are poor (Amann et al., 2000). Presence of a proximal cytoplasmic droplet is indicative of sperm immaturity (the droplet usually migrates along the mid-piece during sperm maturation in the ductus epididymidis). Wood et al. (1986) showed that the proportion of coiled tails and proximal droplets, indicators of sperm maturity, provided the best prediction of fertility in frozen semen, thus requesting a careful consideration of their presence.

Several studies have shown that immature bulls have high percentages of proximal droplets in the peri-pubertal period, but that rapidly decline to normal levels

thereafter (Evans et al., 1995; Johnson et al., 1998; Arteaga et al., 2001; Padrik and Jaakma, 2002), indicating a relationship to the age of the sires. All bulls included in the present study were within such age interval that levels of proximal cytoplasmic droplets were not affected by age. This confirms previous findings that older bulls display low, repeatable percentages of proximal droplets (Söderquist et al., 1996).

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.

## Acknowledgements

The excellent laboratory help provided by Mrs Annika Rikberg and Karin Selin-Wretling at the Sperm Laboratory of the Division of Comparative Reproduction, Obstetrics and Udder Health (ex Dept of Obstetrics and Gynaecology), SLU is deeply acknowledged. Partial financial support was provided by the Swedish Farmers' Foundation for Research in Agriculture (SLF), Stockholm.

## References

- Amann, R.P., Seidel, G.E. and Mortimer, R.G., 2000. Fertilizing potential in vitro of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. *Theriogenology* **54**, 1499–1515.
- Arteaga, A., Baracaldo, M. and Barth, A.D., 2001. The proportion of beef bulls in western Canada with mature spermograms at 11 to 15 months of age. *Canadian Veterinary Journal* **42**, 783–787.
- Bane, A., 1952. A study on the technique of hemocytometric determination of sperm motility and sperm concentration in bull semen. *Cornell Veterinarian* **42**, 518-531.
- Bane, A., 1961. Acrosomal abnormality associated with sterility in boar. *Proc. 4<sup>th</sup> Int. Congr. Anim. Reprod. & A.I., The Hague, IV*, 810-817.
- Barth, A.D., 1997. Evaluation of potential breeding soundness of the bull. In: Youngquist, R.S., Editor, 1997. *Current Therapy in Large Animal Theriogenology*, WB Saunders Co, Philadelphia, pp. 222–236.
- Barth, A.D., Bowman, P., Bo, B. and Mapletoft, R.J., 1992. Effect of narrow sperm head shape on fertility in cattle. *Canadian Veterinary Journal* **33**, 31–39.
- Blom E., 1977. Sperm morphology with reference to bull infertility. *First All-India symposium on Animal Reproduction, Ludhiana*, pp. 61–81.
- Blom, E., 1983. Pathological conditions in the genital organs and in the semen as grounds for rejection of breeding bulls for import and export to or from Denmark. *Nordisk Veterinaermedicin* **35**, 05–130.
- Chenoweth, P.J., 1980. Bull fertility. *Modern Veterinary Practice* **61**, 987–991.

- Coulter, G.H. and Kozub, G.C., 1989. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. *Journal of Animal Science* **67**, 1757–1766.
- Evans, A.C.O., Davies, F.J., Nasser, L.F., Bowman, P. and Rawlings, N.C., 1995. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. *Theriogenology* **43**, 569–578.
- Fitzpatrick, L.A., Fordyce, G., McGowan, M.R., Bertram, J.D., Doogan, V.J., Faveri, J.de., Miller, R.G. and Holroyd, R.G., 2002. Bull selection and use in northern Australia. Part 2. Semen traits. *Animal Reproduction Science* **71**, 39–49.
- Foote, R.H., Seidel, G.E. Jr, Hahn, J. and Berndtson, W. E. 1977 Seminal quality, spermatozoal output, and testicular changes in growing Holstein bulls. *Journal of Dairy Science* **60**, 85-88.
- Gotschall, G.S. and Mattos, R.C., 1997. Findings of andrological examinations of *Bos taurus* and *Bos indicus* bulls. *Revista Brasileira de Reproducao Animal* **21**, pp. 25–28.
- Gustafsson, B., 1966. Luminal contents of bovine epididymis under conditions of reduced spermatogenesis, luminal blockage and certain sperm abnormalities. *Acta Vet. Scand.*, Suppl. 17, Thesis, Stockholm.
- Hallap, T., Håård, M., Jaakma, U., Larsson, B. and Rodriguez–Martinez, H., 2004. Variations in quality of frozen –thawed semen from Swedish and white AI sires at 1 and 4 years of age. *International Journal of Andrology* **27**, 166-171.
- Hancock, J.L., 1957. The morphology of Boar spermatozoa. *J. R. Microsc. Soc.*, **76**, 84-97.
- Holroyd, R.G., Doogan, V.J., Faveri, J.de, Fordyce, G., McGowan, M.R., Bertram, J.D., Vankan, D.M., Fitzpatrick, L.A., Jayawardhana, G.A. and Miller, R.G., 2002. Bull selection and use in northern Australia. 4. Calf output and predictors of fertility of bulls in multiple-sire herds. *Animal Reproduction Science* **71**, 67–79.
- 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 Kazimirow, E., 1997. Morphological characters of bull semen and conception rate. *Medycyna Weterynaryjna* **33**, 561–563.
- Johnson, K.R., Dewey, C.E., Bobo, J.K., Kelling, C.L. and Lunstra, D.D., 1998. Prevalence of morphologic defects in spermatozoa from beef bulls. *Journal of the American Veterinary Medical Association* **213**, 1468–1471.
- Lagerlöf, N., 1934. Morphologische Untersuchungen Über Veränderungen im Spermabild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilität. *Acta Pathologica et Microbiologica Scandinavica* Suppl. **19**, Thesis, Stockholm.
- Larsen, R.E., Littell, R., Rooks, E., Adams, E.L., Falcon, C. and Warnick, A.C., 1990. Bull influences on conception percentage and calving date in Angus, Hereford, Brahman and Senepol single-sire herds. *Theriogenology* **34**, 549–568.
- Linford, E., Glover, F.A., Bishop, C. and Stewart, D.L., 1976. The relationship between semen evaluation methods and fertility in the bull. *Journal of Reproduction and Fertility* **47**, 283-291.
- Meyer, R.A. and Barth, A.D., 2001. Effect of acrosomal defects on fertility of bulls used in artificial insemination and natural breeding. *Canadian Veterinary Journal* **42**, 627–634.
- Morstin, J. and Courot, M., 1974: Ultrastructure des spermatozoides de taureaux de differente fecundance. Morphologie ultrastructurelle, glucoproteines acrosomique et

- membranaires, charges negatives de surface. *Annales de Biologie Animale, Biochimie et Biophysique* **14**, 581-590.
- Nöthling, J.O. and Arndt, E.P., 1995. Fertility of two bulls with poor sperm morphology. *Journal of the South African Veterinary Association* **66**, 74–76.
- Padrik, P. and Jaakma, U., 2002. Sperm morphology in Estonian Holstein dairy bulls, factors affecting it and relation to fertility. *Agraarteadus* **13**, 243–256.
- Pangewar, G.R. and Sharma, R.D., 1989. Physical and biochemical characteristics of semen in relation to fertility of Holstein-Friesian bulls. *Indian Veterinary Journal* **13**, 21–26.
- Parkinson, T.J., 2004. Evaluation of fertility and infertility in natural service bulls. *The Veterinary Journal* **168**, 215-229.
- Parkinson, T.J., 2004. Evaluation of fertility and infertility in natural service bulls. *The Veterinary Journal* **168**, 215-229.
- Petac D. and Kosec, M., 1989. Some morphological characters of bull spermatozoa and their effect on fertility. *Zbornik Biotehniške Fakultete Univerze Edvarda Kardelja v Ljubljani, Veterinarstvo* **26**, pp. 65–72.
- Rodriguez-Martinez, H., 2003. Laboratory semen assessment and prediction of fertility: still utopia? *Reproduction in Domestic Animals* **38**: 312-318.
- Saacke, R.G., Dalton, J., Nadir, S., Bame, J. and Nebel, R.L., 1988. Spermatozoal characteristics important to sperm transport, fertilization and early embryonic development. In Lauria A, Gandolfi F, Enne G, Gianaroli L, eds “Gametes: Development and function” Serono Symposia Milano, **1**, 320-335.
- Saacke, R.G., Nadir, S., Dalton, J., 1995. Assessing bull fertility. In: *Proceedings of the Society for Theriogenology*, 73.
- Söderquist, L., Janson, L., Haard, M. and Einarsson, S., 1996. Influence of season, age, breed and some other factors on the variation in sperm morphological abnormalities in Swedish dairy A.I. bulls. *Animal Reproduction Science* **44**, 91–98.
- Söderquist, L., Janson, L., Larsson, K. and Einarsson, S., 1991. Sperm morphology and fertility in AI bulls. *Journal of Veterinary Medicine* **38**, 534–543.
- Spitzer J.C., Hopkins, F.M., Webster, H.W., Kirkpatrick, F.D. and Hill, H.S., 1988. Breeding soundness examination of yearling beef bulls. *Journal of the American Veterinary Medical Association* **193**, 1075–1079.
- Stålhammar, E.M., Janson, L. and Philipson, J., 1994. Genetic studies on fertility in AI bulls. II. Environmental and genetic effects on non-return rates of young bulls. *Animal Reproduction Science* **34**, 13-27.
- Thibier M, Wagner H-G., 2000. World statistics for artificial insemination in cattle. *Proc 14<sup>th</sup> ICAR Stockholm* **2**, 76 (15:2).
- Thundathil, J., Palasz, A.T., Mapletoft, R.J. and Barth, A.D., 1999. An investigation of the fertilizing characteristics of pyriform-shaped bovine spermatozoa. *Animal Reproduction Science* **57**, 35–50.
- Thundathil, J., Meyer, R., Palasz, A.T., Barth, A.D. and Mapletoft, R.J., 2000. Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Theriogenology* **54**, 921–934.
- 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 Veterinarian* **10**, 87-94.

- Wilmington, J.A., 1981. Some investigations into the effect of sperm morphology on the fertility of semen used for artificial insemination. In: *Proceedings of the Association of Veterinary Teachers and Research Workers*, Scarborough, pp.1-11.
- Wood, P.D.P., Foulkes, J.A., Shaw, R.C. and Melrose, D.R., 1986. Semen assessment fertility and the selection of Hereford bulls for use in AI. *Journal of Reproduction and Fertility* **76**, 783-795.

## **General Conclusions**

- There is a relationship between sperm morphology and fertility after AI in progeny-tested dairy bull sires.
- Sperm head morphology appears to be the predominant variable in this relation.
- The results found that a level of >10% of morphologically abnormal sperm heads is a valuable indicator for this relationship.
- It is important that routine, frequently done screenings of sperm morphology in AI-stud bull sires should be carried out during their entire active life.
- Owing to the presence of age-dependent, and breed variations, sperm morphology should be studied in a larger number of progeny-tested AI bulls, over a longer period.

## **Final prospects**

Since sperm head abnormalities are to be primarily considered as uncompensable, studies should be performed to determine the extent they compromise fertility, e.g. what threshold is to be considered risk for a decreased fertility in terms of proportions of the abnormalities and the number of spermatozoa affected. Long-lasting, longitudinal studies of sperm morphology screening are, therefore, advisable.

## General references

- Almquist JO, Amann RP, 1976: Reproductive capacity of dairy bulls. Pubertal characteristics and post pubertal changes in production of semen and sexual activity of Holstein bulls ejaculated frequently. *Journal of Dairy Science* **59**, 98698
- Almquist, J. O., P. J. Glantz, H. E. Shaffer. 1949. The effect of a combination of penicillin and streptomycin upon the livability and bacterial content of bovine semen. *Journal of Dairy Science* **32**, 183–190.
- Bailey JL, Robertson L, Buhr MM, 1994: Relations among *in vivo* fertility, computer-analysed motility and Ca<sup>++</sup> influx in bovine spermatozoa. *Canadian Journal of Animal Science* **74**, 53–58.
- Ballachey, B. E., D. P. Evenson, R. G. Saacke. 1988. The sperm chromatin structure assay: relationship with alternate tests of semen quality and heterospermic performance of bulls. *Journal of Andrology* **9**, 109–115.
- Boersma, A., K. Selin-Wretling, R. Stolla, H. Rodriguez Martinez, 2000: Relationship between computer-assisted morphometric sperm analysis and visual subjective analysis of bull sperm heads by different evaluators. *Proc. 14<sup>th</sup> ICAR Stockholm, 2-6 July 2000, I: 91.*
- Davis, I. S., R. W. Bratton, R. H. Foote. 1963. Livability of bovine spermatozoa at 5, –25, and –85°C in tris-buffered and citratebuffered yolk-glycerol extenders. *Journal of Dairy Science* **46**, 333–336.
- Den Daas JH, De Jong G, Lansbergen LM, Van Wagendonk-De Leeuw AM, 1998: The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual dairy bulls. *Journal of Dairy Science* **81**, 1714–1723.
- Ericsson SA, Garner DL, Thomas CA, Downing TW, Marshall CE, 1993: Interrelationship among fluorometric analyses of spermatozoal function, classical semen quality parameters and the fertility of frozen-thawed bovine spermatozoa. *Theriogenology* **39**, 1009–1024.
- Evenson D, Jost L, 2000: Sperm chromatin structure assay is useful for fertility assessment. *Methods in Cell Science* **22**, 169–189.
- Evenson, D. P., Darzynkiewicz, Z., Melamed, M. R. (1982). Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility. *Journal of Histochemistry and Cytochemistry* **30**, 279–80.
- Evenson, D. P., Z. Darzynkiewicz, M. R. Melamed, 1980. Relation of mammalian sperm chromatin heterogeneity of fertility. *Science* **240**, 1131–1134.
- Evenson, D.P., 1999. Alterations and damage of sperm chromatin structure and early embryonic failure. In Jansen. R. and Mortimer, D. (eds), *Towards Reproductive Certainty: Fertility and Genetics Beyond 1999*. Parthenon Publishing Group, New York, pp. 313–329.
- Farrell PB, Presicce GA, Brockett CC, Foote RH, 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. *Theriogenology* **49**, 871–879.
- Foote, R. H., 1998. Artificial Insemination to Cloning: Tracing 50 Years of Research. Published by the author, Ithaca, New York.
- Foote, R. H., 2002. The history of artificial insemination: selected notes and notables. American Society of Animal Science
- Foote, R. H., R. W. Bratton. 1949. The fertility of bovine semen cooled with and without the addition of citrate-sulfanilamide-yolk extender. *Journal of Dairy Science* **32**, 856–861.
- Foote, R. H., R. W. Bratton, 1950. The fertility of bovine semen in extenders containing sulfanilamide, penicillin, streptomycin, and polymyxin. *Journal of Dairy Science* **33**, 544–547.
- Foote, R. H., 1999. Artificial Insemination from the origins up to today. In: V. Russo, S. Dall'Ólio, and L. Fontanesi (eds). *Proc. of the Spallanzani Int. symp., Reggio Emilia, Italy.* 23–67.

- Graham, J. K., Kunze, E., Hammerstedt, R. H. 1990. Analysis of sperm viability, acrosomal integrity and mitochondrial function using flow cytometry. *Biology of Reproduction* **43**, 55-64.
- Gravance, C.G., D.L. Garner, C. Pitt, R. Vishwanath, S.K. Sax-Gravance, P.J. Casey, 1999. Replicate and technician variation associated with computer aided bull sperm head morphometry analysis (ASMA). *International Journal of Andrology* **22**, 77-82.
- Håård, M.C., M.G.H. Håård, 2000. Account of an attempt to estimate the dose response for individual bulls in commercial AI-industry. *Proc. 14<sup>th</sup> ICAR Stockholm, 2-6 July 2000*, **II**: 80 (15:6).
- Hultåns, 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 Agriculturae Scandinavica*, Suppl. 6, Thesis, Stockholm.
- Iritani, A. 1980. Problems of freezing spermatozoa of different species. In: *Proc. 9th Int. Congr. Anim. Reprod. Artif. Insemin., Madrid, Spain* **1**, 115-131.
- Januskauskas A, Gil J, Söderquist L, Rodriguez Martinez H, 2000b. Relationship between sperm response to glycosaminoglycans *in vitro* and non return rates of Swedish dairy AI bulls. *Reproduction in Domestic Animals* **35**, 207-212.
- Januskauskas A, Johannisson A, Rodriguez Martinez H, 2003. Subtle membrane changes in cryopreserved bull semen in relation to sperm viability, chromatin structure and field fertility. *Theriogenology* **60**, 743-758.
- Januskauskas A, Johannisson A, Rodriguez Martinez H, 2001. Assessment of sperm quality through fluorometry and sperm chromatin structure assay in relation to field fertility of frozen-thawed semen from Swedish AI bulls. *Theriogenology* **55**, 947-961.
- Januskauskas A, Johannisson A, Söderquist L, Rodriguez Martinez H, 2000a. Assessment of the fertilizing potential of frozen-thawed bovine spermatozoa by calcium ionophore A23187-induced acrosome reaction *in vitro*. *Theriogenology* **53**, 859-875.
- Januskauskas A, Söderquist L, Haard MC, Lundeheim N, Rodriguez Martinez H, 1996. Influence of sperm number per straw on the postthaw sperm viability and fertility of Swedish Red and White AI bulls. *Acta Veterinaria Scandinavica* **37**, 461-470
- Kjærstad H, Ropstad E, Andersen Berg K, 1993. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. *Acta Veterinaria Scandinavica* **34**, 299-303.
- Lagerlöf, N., 1934. Morphologische Untersuchungen Über Veränderungen im Spermabild und in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilität. *Acta Pathologicae Microbiologicae Scandinavica*, Suppl. **19** Thesis, Stockholm.
- Nishikawa, Y, 1962. Fifty years of artificial insemination of farm animals in Japan. English Bull. 2. Kyoto University, Japan.
- Pace MM, Sullivan JJ, Elliott FI, Graham EF, Coulter GH, 1981. Effects of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of bovine spermatozoa packaged in 0.5 ml French straws. *Journal of Animal Science* **53**, 693-701
- Perry, E. J. (ed.), 1968. *The Artificial Insemination of Farm Animals*. 4th ed. Rutgers University Press, New Brunswick, NJ.
- Philips Z, Barraza-Llorens M, Posnett J, 2000. Evaluation of the relative cost-effectiveness of treatments for infertility in the UK. *Human Reproduction* **15**, 95-106.
- Phillips, P. H., H. A. Lardy, 1940. A yolk-buffer pabulum for the preservation of bull semen. *Journal of Dairy Science* **23**, 399-404.
- Pickett, B. W., W. E. Berndtson, 1974. Preservation of bovine spermatozoa by freezing in straws: A review. *Journal of Dairy Science* **57**, 1287-1301.
- Polge, C., A.U. Smith, A. S. Parkes, 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature (Lond.)* **164**, 666.
- Rodin, I.M., Liptov, V.I, 1936. Artificial insemination of pigs. *Animal Breeding Abstracts* **4**, 205.
- Rodriguez-Martinez, H., Larsson, B., Pertoft, H., Kjellen, L, 1998. GAGs and spermatozoon competence *in vivo* and *in vitro*. In: *Gamete Development and Function* (eds A. Lauria *et al.*), pp. 239-272. Sero Symposium, Rome.

- Saacke, R. G., J. C. Dalton, S. Nadir, R. L. Nebel, J. H. Bame, 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Animal Reproduction Science* **60–61**, 663–677.
- Saacke, R. G., J. M. DeJarnette, J. H. Bame, D. S. Karabinus, S. S. Whitman. 1998. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? *Theriogenology*. **50**, 117–128.
- Saeed, A., Chaudhry, R. A., Khan, I. H., Khan, N. U, 1989. Studies on morphology of buffalo bull semen of different age groups. *Buffalo Journal* **1**, 99–102.
- Sailer BL, Nastasi AJ, Valdez JG, Steinkamp JA, Crissman HA, 1996. Interactions of intercalating fluorochromes with DNA analyzed by conventional and fluorescence lifetime flow cytometry utilizing deuterium oxide. *Cytometry* **24**, 167-173.
- Salisbury, G. W., H. K. Fuller, E. L. Willett, 1941. Preservation of bovine spermatozoa in yolk-citrate diluent and field results from its use. *Journal of Dairy Science* **24**, 905–910.
- Schwartz D, MacDonald PDM, Heuchel V, 1981. On the relationship between the number of spermatozoa and the probability of conception *Réproduction, Nutrition Développement* **21**, 979–988.
- Shannon P, Vishwanath R, 1995. The effect of optimal and suboptimal concentrations of sperm on the fertility of fresh and frozen bovine semen and a theoretical model to explain the fertility differences. *Animal Reproduction Science* **39**, 1–10
- Söderquist, L., 1991. Sperm characteristics and fertility in dairy AI bulls. PhD thesis, Uppsala, Sweden.
- Sørensen, E, 1938. Kunstig Saedeverforing Hos Huspattedyrene. *Kongelig Veterinaer-og Landbøjskole Aarsskrift*, Copenhagen.
- Stålhammar EM, Janson L, Philipsson J, 1994. The impact of sperm motility on non-return rate in preselected dairy bulls. *Reproduction, Nutrition Development* **34**, 37–45.
- Swensson, T., 1993. Något om artificial insemination och dess tillämpning under de första decennierna i Sverige. *Nordisk Veterinärförening för Husdjursreproduktion Svenska Sektionen*.
- Thibier M, Wagner HG, 2000 World statistics for artificial insemination in cattle. *Proc 14th International Congress of Animal Reproduction, Stockholm*, **2**, 76
- Thundathil, J., Meyer, R., Palasz, A.T., Barth, A.D., Mapletoft, R.J., 2000. Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Theriogenology* **54**, 921–934.
- Thundathil, J., Palasz, A.T., Mapletoft, R.J., Barth, A.D., 1999. An investigation of the fertilizing characteristics of pyriform-shaped bovine spermatozoa. *Animal Reproduction Science* **57**, 35–50.
- Thundathil, J.; Kastelic, J.P.; Mapletoft, R.J., 1998. The effect of estradiol cypionate (ECP) on ovarian follicular development and ovulation in dairy cattle. *Canadian Journal of Veterinary Research* **61**, 314-316.
- Uwland J, 1984. Verband tussen het aantal geïnsemineerde zaadcellen en de bevruchttingsresultaten bij melkvee *Tijdschrift voor Diergeneeskunde* **109**, 405–412.
- Van Vleck, L. D, 1981. Potential genetic impact of artificial insemination, sex selection, embryo transfer, cloning and selfing in dairy cattle. In: B. C. Brackett, G. E. Seidel, Jr., and S. M. Seidel (ed.) *New Technologies in Animal Breeding*. Academic Press, New York.
- Vishwanath, R, 2003. Artificial insemination: the state of the art. *Theriogenology* **59**, 571-584
- Zhang BR, Larsson B, Lundeheim N, Rodriguez-Martinez H, 1998. Sperm characteristics and zona pellucida binding in relation to field fertility of frozen–thawed semen from dairy AI bulls. *International Journal of Andrology* **21**, 207–216.

## Acknowledgements

This study was carried at the Division of Comparative Reproduction, Obstetrics and udder health (ex. Dept of Obstetrics and Gynaecology), Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

I would like to express my deeply sincere gratitude to all those direct or indirect support, guidance, and wishes made it possible for me to complete my studies. I also want to express my sincere thanks especially to all the following people who have contributed to this work and guided me during my studies:

Professor Heriberto Rodriguez Martinez, head of the Division of Reproduction, my scientific supervisor, for his optimism and excellent guidance. For his patience while correcting my manuscript several times and making it best with recent knowledge and for his dedication, despite a very busy programme of his own. I really appreciate your helpful understanding nature. Thanks for your encouragement and strong guidance.

Associate professor Nils Lundheim, my co-author, Thank you for the great help you gave me in introducing me to the world of statistics, in which I had not previously worked. Thanks for letting me to know how easy this subject is and for solving the problems I used to face during the work.

Dr.Margareta Håård, my co-author for her help in supplying me with all the data needed. I would like to thank you for the nice introduction you gave me about Svensk Avel during my visit to the company. Also, for revising my manuscript and answering all the questions.

To the people at the Swedish international program on animal reproduction (SIPAR) for their superbly organized Master of Science program: Dr. Karin Östensson for giving me the great opportunity to study in Sweden at SLU. I also want to extend the great appreciation to Marie Sundberg for making this MSc programme environment such comfortable and for her best care of the MSc Programme for international students at SLU. You were so good at practical matters.

Many thanks to all the staff of the Division, for being kind to me. Particular thanks to Annika Rikberg and Karin Selin-Wretling for their expertise, for the innumerable assistance in many laboratory matters and for translating for me many Swedish sentences that I wished to learn.

Kjell- åke Ahlin, for technical assistance with my computer and Kjell-Ove Eklund for technical help and the provision of my bicycle.

Birgitta Berner for their excellent secretarial help.

All former and present PhD and master students at the department for creating pleasant atmosphere:

the MSc-students, Dr.Inoka Perris, Dr. Iftkhar Ali, and Dr. Charles Lay, Dr.Urancheming for nice friendship during our courses and social life. My scientific colleagues, I am very thankful for your research assistance, especially in the final spurt of my studies. PhD students, Dr. Yoseph Mekasha and Dr. Fikre Lobaggo and Dr.Jatesada Jiawkanon for help with our common statistical problems. Dr. Ann-Sofi Bergqvist, Dr.Yaohong Zhu, Dr.Ylva Brandt, Dr. Linda Spjuth, Dr. Ulrika Grönlund, Dr. Ulrika Hermansson, and all other colleagues for making a very nice harmonic pleasant atmosphere here at the department.

All the library staff at KC and the main library for their excellent service.

All the Arabic families and friends in Uppsala and Stockholm for sharing experiences, having fun and enjoying life together which helped me a lot in progressing my steps easily in the long way of my studies.

No deepest word in my mind can I express to my father Associate Professor Dr. Khalaf Al-makhzoomi and my mother Mona Sulieman for encouraging me in this nasty world of science and private life and to organize my wedding ceremony during my fast visit to my dear country (The Hashemite kingdom of Jordan). Also, my dearest brothers Alla and Mohammad and my sisters Professor Ibtihal, Batool and Afya whose consistent inspiration, scientific encouragements and endless love helped me to make all my efforts fruitful.

Last, but not least, I wish to thank, with all my heart, my sweet beautiful wife FATEN Al-Yabroudi for her incredible support and endless love.