ELSEVIER

Animal Reproduction Science 97 (2007) 1–11

ANIMAL REPRODUCTION SCIENCE

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Effects of sperm concentration at semen collection and storage period of frozen semen on dairy cow conception

T. Haugan [a,*], Y.T. Gröhn [b], E. Kommisrud [c], E. Ropstad [a], O. Reksen [a]

[a] *Department of Production Animal Clinical Sciences, The Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N-0033 Oslo, Norway*

[b] *Section of Epidemiology, Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

[c] *Geno Breeding and AI Association, N-2326 Hamar, Norway*

Received 22 July 2005; received in revised form 1 December 2005; accepted 28 December 2005
Available online 7 February 2006

Abstract

The present study was based on data obtained from artificial inseminations (AIs) performed with cryopreserved semen from elite bulls used in the Norwegian breeding program. Semen was diluted to standardize the number of spermatozoa to 18 million per AI dose. The aim of the study was to investigate whether the net sperm concentration at semen collection and the storage period in liquid nitrogen have any effect on probability of conception in dairy cattle. We demonstrated that the natural range of sperm concentration at semen collection within some of the bulls was associated with the probability of conception. However, no primary trend among bulls was found on the effect of sperm concentration at semen collection. This appears to be due to differences among bulls in their response to the dilution ratio of seminal plasma to extender. The effect of storage time was investigated in semen that had been stored between 1000 days and 2400 days in AI straws in liquid nitrogen at the AI center. Our findings showed that use of semen with the longest storage period, i.e. 1951–2400 days, resulted in a more than one percentage point lower probability of conception than semen with a shorter storage period. In conclusion, the net sperm concentration at semen collection, which affects the dilution ratio of seminal plasma to extender, should be considered individually among bulls to achieve optimal reproductive performance. Furthermore, this study gives support to the idea

* Corresponding author. Present address: Team Semin, P.O. Box 8146 Dep., N-0033 Oslo, Norway. Street address: Ullevålsveien 72, Building 13, 0454 Oslo, Norway. Tel.: +47 22964862; fax: +47 22597081.
E-mail address: tommy.haugan@veths.no (T. Haugan).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2005.12.010

that a measurable degree of damage to the spermatozoa could occur during the preservation time in liquid nitrogen.

Keywords: Cryopreservation; Storage period; Sperm concentration; Reproductive performance

1. Introduction

The number of viable bovine spermatozoa deposited in the female reproductive tract has been shown to influence fertilizing ability up to an upper threshold level (Pace et al., 1981; Schenk et al., 1987; Gerard and Humblot, 1991). When using artificial insemination (AI), the semen is diluted in an extender and the number of spermatozoa per AI dose is standardized. This reduces the direct advantage of high sperm output in the ejaculate on fertility potential. However, when we dilute to standard cell numbers, we dilute seminal plasma as well. The final dilution ratio of seminal plasma to extender in the AI straws depends on the sperm concentration at semen collection. The extender used for cryopreservation of semen contains cryoprotectants (such as glycerol and egg yolk), substances to maintain the osmolarity, energy source (such as glucose or fructose), and enzymes and antibiotics that are essential for maintaining the viability of the spermatozoa during cooling, freezing and thawing (Vishwanath and Shannon, 2000; Holt, 2000). On the other hand, the seminal plasma may also contribute components, like proteins, that positively affect sperm viability and (or) cryosurvival (Miller et al., 1990; Henricks et al., 1998; Kohsaka et al., 2003). Conversely, other studies have shown that seminal plasma could be detrimental to the fertilizing ability of spermatozoa (Shannon, 1965; Shivaji and Bhargava, 1987; Way et al., 2000). The composition of seminal plasma is not homeostatic and varies among species, but also among individuals and among ejaculates from the same animal (Garner and Hafez, 2000). Considering that both the semen extender and the seminal plasma contain components that affect the spermatozoa, there might be an optimal dilution ratio, which differs among bulls. Thus, when standardized, AI doses are used; it is reasonable to question whether the sperm concentration at the time of semen collection (i.e. in the ejaculate) is associated with probability of conception.

Another factor to consider is that the sperm concentration in the ejaculate serves as one of the criteria of the semen characteristics to qualify fertile males for breeding purposes (Graffer et al., 1988). Significant bull differences in sperm concentration at semen collection have been shown in previous studies (Graffer et al., 1988; Seidel and Foote, 1969; Shelke and Dhami, 2001). Sperm concentration in semen collection could be considered as an initial indicator of semen quality in semen used for cryopreservation (Shelke and Dhami, 2001; Belorkar et al., 1988). A positive correlation between sperm concentration at semen collection and motility has been reported (Everett et al., 1978; Mathevon et al., 1998). However, this may partly rely on overestimation of motility in more concentrated samples (Everett et al., 1978). Overall, the literature is scarce concerning whether sperm concentration at the time of semen collection could be an indicator of fertilization potential among normal fertility sires.

Cryopreservation in liquid nitrogen (where the temperature is $-196\,^{\circ}C$) is a technique that makes long-term storage of spermatozoa possible. This is of high practical importance for breeding programs in domestic animals, and the technique is also used to maintain the genetic diversity and establishment of gene-banks (Jalme et al., 2003; Shivaji et al., 2003). The success of fertilization with use of frozen–thawed spermatozoa varies considerably between species and among individ-