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Effect of different thawing rates on post-thaw sperm viability, kinematic parameters and motile sperm subpopulations structure of bull semen

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Abstract

The aim of the present study was to evaluate three thawing rates for bull semen frozen in 0.25-ml straws: placing the straws in a water bath at 37 °C for 40 s, at 50 °C for 15 s or at 70 °C for 5 s. In a first experiment, the three thawing rates were compared in relation to post-thaw sperm motility, determined subjectively, and sperm plasma and acrosomal membrane integrity, examined by flow cytometry, after 0 and 5 h of incubation at 37 °C. In a second experiment, the three thawing rates were evaluated based on post-thaw sperm motility, determined using a CASA system, after 0 and 2 h of incubation at 37 °C. In addition, for the motile spermatozoa, the individual motility descriptors were analysed using a multivariate clustering procedure to test the presence of separate sperm subpopulations with specific motility characteristics in the thawed bull semen samples. Finally, it was investigated if the thawing rate had any influence on the relative frequency distribution of spermatozoa within the different subpopulations. In terms of overall post-thaw motility or plasma and acrosomal sperm membrane integrity there were no significant differences between the three thawing methods evaluated. The statistical analysis clustered all the motile spermatozoa into four separate subpopulations with defined patterns of movement: (1) moderately slow and progressive sperm (27%); (2) "hyperactivated-like" sperm (15.4%); (3) poorly motile non-progressive sperm (34.3%); (4) fast and progressive sperm (23.3%). The thawing rate had no significant influence on the frequency distribution of spermatozoa within the four subpopulations, but there was a significant effect ($P < 0.05$) of the interaction between thawing rate and incubation time. Higher proportions of spermatozoa with fast and progressive movement were observed after 2 h of post-thaw incubation when the thawing was at the faster

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rates (35 °C/40 s: 8.3%, 50 °C/15 s: 18.1% and 70 °C/5 s: 16.5%). Whether this subtle difference might affect to the *in vivo* fertility of the thawed bovine semen is not known.

Keywords: Bull semen; Thawing rates; Kinematic parameters; Sperm subpopulations

1. Introduction

Artificial inseminations in dairy cattle are mainly done with frozen-thawed semen. The rate of thawing significantly affects the sperm survival, and the appropriate thawing rate is thought to be influenced by numerous factors of the cryopreservation procedure such as type of extender, glycerol concentration, packaging method and freezing rate (Rodriguez et al., 1975; Robbins et al., 1976).

According to the bifactorial theory of cryoinjury proposed by Mazur (1965), optimum cell survival requires that the freezing rate be paired with the thawing rate. Although experimental evidence suggests that this theory applies to spermatozoa (reviewed by Watson, 1979), several studies on bull sperm cryopreservation failed to confirm any relationship between freezing and thawing rates. Almquist and Wiggin (1973a,b) and Robbins et al. (1973) concluded that freezing rates are relatively unimportant in affecting post-thaw motility or acrosomal integrity if the semen is thawed rapidly. On the contrary, Rodriguez et al. (1975) found that a slow rate of freezing resulted in low sperm survival independently of the thawing rate.

A variety of studies evaluating a range of different thawing rates for bull semen frozen in straws (Aamdal and Andersen, 1968; Almquist and Wiggin, 1973a,b; Rodriguez et al., 1975; Robbins et al., 1976; Dhami et al., 1992; Nur et al., 2003), have generally concluded that the more rapid thawing rates result in better sperm motility and acrosomal integrity. Even though comparable conception rates may be achieved if sperm dosage is sufficiently high to maintain above threshold sperm numbers, slow thawing rates (as those achieved during thawing at ambient temperature, in a shirt pocket, or in water bath at 20 °C or less) usually result in reduced sperm survival and are not recommended (DeJarnette et al., 2000; DeJarnette and Marshall, 2005). In general, unless specific recommendations are given, bovine semen frozen in straws, irrespective of extender type and cooling rate, is recommended to be thawed in a water bath at 33–35 °C for 30–40 s (DeJarnette et al., 2000; DeJarnette and Marshall, 2005). However, a number of studies have shown that thawing temperatures as high as 60–80 °C could further improve post-thaw motility (Rodriguez et al., 1975; Senger, 1980; Dhami et al., 1992; Nur et al., 2003). Although the use of such a high temperature is far from being a practical method of thawing the straws, especially in field conditions, it appears to cause a lower degree of cellular damage, yet the magnitude of this effect is still uncertain.

Most of the previous studies designed to evaluate thawing procedures for bull spermatozoa were based on the subjective microscopic assessment of post-thaw sperm motility, morphology or membrane integrity. Currently, more objective and precise measurements on functional and structural characteristics of spermatozoa can be achieved using flow cytometry and CASA systems. The application of new technologies available for semen analysis to the re-evaluation of some controversial aspects of the cryopreservation protocols such as, for example, if there is a real advantage in using thawing temperatures higher than 35 °C could allow us to gain new and valuable information.

Substantial amount of data supports the hypothesis that any mammalian ejaculate constitutes a heterogeneous population of spermatozoa within which functionally different subpopulations coexist (Abaigar et al., 1999). Studies carried out by several researchers in mammalian ejaculates