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Identification of sperm subpopulations with defined motility characteristics in ejaculates from Holstein bulls: Effects of cryopreservation and between-bull variation

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Abstract

The aims of the present study were: (1) to determine the existence of sperm subpopulations with specific motility characteristics in fresh ejaculates from Holstein bulls, (2) to investigate the effects of semen cryopreservation and post-thaw incubation on the distribution of spermatozoa within the different subpopulations, and (3) to evaluate the existence of between-bull variation in the sperm subpopulations structure of fresh and frozen-thawed semen. Six ejaculates were collected from each of 9 Holstein bulls and cryopreserved following a standard protocol. Overall sperm motility and the individual kinematic parameters of motile spermatozoa, determined using a CASA system, were evaluated before freezing and after 0, 2 and 4 h of post-thaw incubation at 37 °C. Data from 16,740 motile spermatozoa, defined by VCL, VSL, VAP, LIN, STR, WOB, ALH and BCF, were analysed using a multivariate clustering procedure to identify and quantify specific subpopulations within the semen samples. The statistical analysis clustered all the motile spermatozoa into four separate subpopulations with defined patters of movement: Subpopulation (Subp. 1) moderately slow but progressive spermatozoa (23.2%), (Subp. 2) highly active but non-progressive spermatozoa (16.0%), (Subp. 3) poorly motile non-progressive sperm (35.5%), and (Subp. 4) highly active and progressive sperm (25.3%). Subpopulations 2 and 4 significantly ($P < 0.01$) decreased during cryopreservation and post-thaw incubation (Subp. 2: 21.1%, 18.1%, 8.7% and 5.9%; and Subp. 4: 34.1%, 20.6%, 15.2% and 7.3%, respectively, for fresh, 0, 2 and 4 h post-thaw) whereas Subp. 3 significantly ($P < 0.01$) increased (10.7%, 27.2%, 27.2% and 30.7%, respectively, for fresh, 0, 2 and 4 h post-thaw). The frequency distribution of spermatozoa within subpopulations was quite similar for the 9 bulls, either in fresh or frozen–thawed semen, and differences among bulls were mainly due to differences in the Subp. 4. Significant correlations ($P < 0.01$)

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were found between the proportions of spermatozoa assigned to Subp. 4 in the fresh ejaculates and those in frozen-thawed semen after 0 ($r = 0.473$), 2 ($r = 0.513$) and 4 h post-thaw ($r = 0.450$). This indicated that the ejaculates with the highest subpopulations of rapid and progressive sperm were also the most resistant to cryopreservation and showed the best post-thaw sperm longevity.

Keywords: Bull semen; Cryopreservation; Kinematic parameters; Sperm subpopulations

1. Introduction

Substantial amount of data supports the hypothesis that any mammalian ejaculate constitutes a heterogeneous population of spermatozoa within which functionally different subpopulations coexist (Curry and Watson, 1994; Harrison, 1996; Holt, 1996; Abaigar et al., 1999). Such heterogeneity makes it possible for the female reproductive tract to exert multiple selective processes which will finally reduce a population of several millions to a few competent spermatozoa (Holt and Van Look, 2004). This concept of sperm heterogeneity has been taken into consideration by many researchers, and various computerized and laboratory methods for sperm quality assessment have been developed, in an attempt to distinguish the subpopulation of potentially competent spermatozoa among the whole sperm population (Evenson et al., 1980; Sailer et al., 1996; Thomas et al., 1997; Abaigar et al., 1999; Thurston et al., 2001; Peña et al., 2005; Rubio-Guillén et al., 2007).

Different sperm subpopulations have been identified in mammalian ejaculates on the basis of the motility characteristics displayed by individual spermatozoa. Studies carried out by several researchers on fresh and frozen-thawed semen from species as diverse as marmosets (Holt, 1996), gazelles (Abaigar et al., 1999, 2001), boars (Abaigar et al., 1999; Quintero-Moreno et al., 2004; Cremades et al., 2005; Rivera et al., 2005, 2006), stallions (Quintero-Moreno et al., 2003), dogs (Nuñez-Martínez et al., 2006a,b) or rabbits (Quintero-Moreno et al., 2007) have demonstrated that, using CASA systems, it is possible to identify and quantify different sperm subpopulations with specific patterns of movement. This can be achieved by using different procedures of multivariate clustering analysis applied to the CASA-derived kinematic parameters obtained for each individual spermatozoon in a semen sample.

In mammals, sperm motility is important for sperm transport within the female reproductive tract and for egg penetration. Distinct sperm populations showing forward progressive motility or, in contrast, non-progressive patterns of movement will have different probability to cross the utero-tubal junction and enter the oviduct (Gaddum-Rosse, 1981; Olds-Clarke, 1986; Shalgi et al., 1992; Scott, 2000). Furthermore, the number of spermatozoa able to traverse the barriers of the female reproductive tract to reach the fertilization site has been demonstrated to be possitively associated with the fertility of several domestic species (Overstreet and Adams, 1971; Hunter and Wilmut, 1984; Weitze et al., 1988; DeJarnette et al., 1992; Nadir et al., 1993). Therefore, the identification of sperm subpopulations with a preferential ability to reach the female oviducts might be of utmost importance to improve the accuracy of the sperm quality assessments, and proportions of motile but probably ineffective spermatozoa (non progressive, poorly motile or hyperactivated sperm) could be precisely quantified.

Artificial inseminations in dairy cattle are mainly done with frozen-thawed semen. The cryopreservation process not only induces a loss of sperm viability but also impairs the functionality of the surviving spermatozoa, which accounts for the lower fertilizing capacity of the frozen-thawed