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Assessment of *in vitro* sperm characteristics in relation to fertility in dairy bulls

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Abstract

The performance of frozen-thawed spermatozoa from 10 Holstein bulls in a range of *in vitro* diagnostic tests and the relationship with adjusted *in vivo* fertility data was determined. The tests included an assessment of motility (subjective and computer-assisted), morphology, concentration, viability, acrosomal and chromatin integrity conducted immediately post-thaw and after swim-up, in conjunction with membrane status (CTC staining) and migration in an artificial cervical mucus. Adjusted *in vivo* fertility correlated with subjectively assessed post-thaw motility ($r = 0.672$, $p = 0.033$), post-thaw straight-line velocity ($r = 0.636$, $p = 0.048$), post-thaw sperm morphology ($r = -0.762$, $p = 0.010$), post-thaw sperm viability ($r = 0.635$, $p = 0.048$), the concentration of spermatozoa after swim-up ($r = 0.649$, $p = 0.042$), sperm morphology after swim-up ($r = -0.687$, $p = 0.028$), the number of spermatozoa migrating 10 mm into artificial cervical mucus ($r = 0.632$, $p = 0.050$) and the distance migrated by the vanguard spermatozoon in artificial mucus ($r = 0.701$, $p = 0.024$). A stepwise regression analysis identified tests which, when combined, produced models with a strong correlation ($R^2 > 0.9$) to fertility.

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Keywords: Sperm quality; Fertility; Bulls; Non-return rate; Cattle

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1. Introduction

The fertility of a young bull is generally evaluated after its frozen-thawed semen is used in a large-scale artificial insemination (AI) program. This method is expensive, time-consuming and only allows a limited number of bulls to be tested at any given time. Consequently, it would be of great benefit to the cattle industry to develop a simple, accurate and reliable *in vitro* method of assessing the potential fertility of bulls based on an analysis of their semen.

Traditional *in vitro* evaluation of semen quality involves the subjective assessment of motility, an estimate of the proportion of spermatozoa with normal morphology and an estimate of the concentration of spermatozoa in a unit dose. While these tests set minimum standards for semen used for AI, they have limited value for predicting the subsequent fertility of the sample (Rodriguez-Martinez, 2000). As a result, attention has been directed towards the assessment of other aspects of semen quality as predictors of fertility, such as viability (Januskauskas et al., 2000; Alm et al., 2001), acrosomal integrity (Correa et al., 1997), membrane status (Thundathil et al., 1999; Januskauskas et al., 2000), DNA integrity (Ballachey et al., 1987; Januskauskas et al., 2001), membrane proteins (Parent et al., 1999) and the ability of spermatozoa to swim-up (Zhang et al., 1998; Januskauskas et al., 2000). While some success has been achieved, few single *in vitro* sperm parameters show a reliable and repeatable correlation with field fertility (Rodriguez-Martinez, 2000). In an attempt to address this issue the performance of spermatozoa in *in vitro* tests which assess multiple sperm traits have been used, such as *in vitro* fertilisation, or the performance of spermatozoa in a number of *in vitro* tests have been combined to produce a model.

In the present study, a range of *in vitro* diagnostic tests were performed on a group of bulls ($n = 10$) with well established fertility. A wide range of *in vitro* diagnostic tests were chosen including traditional semen assessments; subjective assessment of motility, morphology assessment and concentration in a unit dose, in conjunction with computer-assisted semen analysis, acrosomal integrity, chromatin integrity, membrane status, the ability of sperm to perform in a swim-up assay and penetration of artificial cervical mucus. These *in vitro* diagnostic tests were chosen in an attempt to identify tests with a high correlation with fertility that would fit into the routine evaluation of a bull in an AI centre. Bulls chosen for inclusion in the study had semen parameters within ranges considered acceptable for use for AI. Despite this they displayed a wide range of fertility, suggesting that currently used parameters do not accurately predict fertility. The data were statistically analysed to determine if a correlation existed between individual and combinations of *in vitro* tests and adjusted *in vivo* fertility.

2. Materials and methods

2.1. Reagents and media

Unless otherwise stated, all media components were purchased from Sigma–Aldrich (St. Louis, MO, USA). The culture medium used for swim-up was modified Tyrode's (Sp-TALP) containing 100 mM NaCl, 3.1 mM KCl, 0.4 mM $MgCl_2$, 2 mM $CaCl_2$, 0.3 mM KH_2PO_4, 25 mM Na HCO_3, 10 mM Na-HEPES, 1 mM pyruvic acid (sodium salt), 21.6 mM Na-lactate, 6 mg/ml BSA (Fraction V) described by Parrish et al. (1988). The culture medium used for the sperm migration assay was cryodiluent, lacking glycerol and egg-yolk (TCF; 200 mM Tris, 67 mM citric acid, 56 mM fructose and 1% (v/v) BSA) and was chosen in an attempt to minimise the effect of medium and dilution on the performance of spermatozoa in the migration assay. The pH of the media used for

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