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ScienceDirect
Theriogenology 76 (2011) 161–167
Theriogenology
www.theriojournal.com

In vitro assessment of sperm from bulls of high and low field fertility

A. Al Naib[a], J.P. Hanrahan[b], P. Lonergan[a], S. Fair[c,*]

[a] School of Agriculture, Food Science and Veterinary Medicine, College of Life Sciences, University College Dublin, Belfield, Dublin 4, Ireland
[b] Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland
[c] Department of Life Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland

Received 18 November 2009; received in revised form 5 October 2010; accepted 10 October 2010

Abstract

The aim of this study was to investigate the reasons for differences in field fertility of bulls following insemination with frozen-thawed semen. The study was carried out in two separate parts over two years and comparisons were made between 5 high and 4 low fertility Holstein Friesian bulls as determined by their either 90 day non-return rate (Year 1) or calving rate (Year 2). Two high fertility Limousin bulls were included in Year 1 for comparative purposes. The ability of sperm from each bull to penetrate artificial mucus was assessed (Year 1 = 7 replicates; Year 2 = 5 replicates). Glass capillary tubes (2 per bull per replicate) were filled with artificial mucus and incubated with sperm stained in 1% Hoechst 33342 for 30 min at 37 °C. The number of sperm were subsequently counted at 10 mm intervals along the tube between 40 and 80 mm markers. Sperm mitochondrial activity of each bull was assessed by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay (4 replicates in each year). Sperm were incubated with MTT for 1 h at 37 °C following which the absorbance of formazan was read using a spectrophotometer. Sperm viability after thawing was assessed for each bull using a live/dead sperm viability kit (Year 1 = 3 replicates; Year 2 = 4 replicates). A minimum of 250 cells were assessed per bull in each replicate and classified as either live or dead. Finally, the ability of sperm to fertilize oocytes *in vitro* and their ability to develop to blastocyst stage embryos were assessed (5 replicates in each year involving 220 to 306 oocytes per bull). Data transformation to normalize residuals was required for mucus sperm penetration (square root) and IVF (cleavage and blastocyst rate) results (arcsin). The mean number of sperm counted at each 10 mm mark between 40 and 80 mm was higher in the high fertility (56.0; 95% CI 39.5 to 75.3) compared to the low fertility (42.9; 95% CI 29.3 to 59.1) Holstein Friesian bulls but the difference did not reach formal significance (P = 0.09). Fertility status had no effect on the ability of sperm to reduce MTT to formazan (mean absorbance 0.34 ± 0.051 and 0.30 ± 0.044) or on the percentage of live sperm per straw (mean 47.3 ± 5.47 and 32.4 ± 4.66) for high and low fertility Holstein Friesian bulls respectively. Oocyte cleavage rate following insemination with sperm from high fertility Holstein Friesian bulls was significantly higher than with sperm from low fertility Holstein Friesian bulls [76.7% (95% CI 60.9 to 89.4) and 55.3 (95% CI 40.4 to 69.7) respectively, P = 0.04]. There was no significant effect of bull fertility on blastocyst rate [34.7% (95% CI 21.1 to 49.6) and 24.2 % (95% CI 14.1 to 36.0) for the high and low fertility Holstein Friesian bulls, respectively; P = 0.2]. In conclusion, sperm from high fertility bulls tended to be more effective in penetrating artificial mucus and to have an increased ability to fertilize oocytes *in vitro*; however, once fertilization occurred subsequent embryo development was not significantly affected by fertility status.

Keywords: Bull semen; Field fertility; Artificial mucus; IVF; MTT

1. Introduction

The introduction of artificial insemination (AI) to the dairy industry in the 1950s revolutionized cattle breeding and has displaced natural service as the preferred method of breeding in most developed countries.

* Corresponding author. Tel.: 00353 61 202548.
E-mail address: sean.fair@ul.ie (S. Fair).

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doi:10.1016/j.theriogenology.2010.10.038

More recent developments in molecular biology have enabled the genomic selection of young elite bulls for inclusion in AI programmes [1]. This technology is now commercially available and coupled with AI allows for faster genetic progress [2]. However, one of the problems of using young bulls in artificial insemination programmes is that, by definition, their field fertility has not been proven. The fertility of a bull has traditionally been evaluated by test inseminations in the field and while this method is considered reliable, it is expensive and time-consuming [3]. Consequently, it would be of benefit to the cattle breeding industry to have an accurate, simple and efficient *in vitro* method of predicting the potential fertility of semen, where aspects such as time, cost and practicability are considered. In order to develop such a test it is first necessary to understand why frozen-thawed semen from some bulls results in a higher pregnancy rate than that from other bulls. Several hypotheses exist as to possible reasons for such differences. Frozen-thawed sperm from low fertility bulls may exhibit an abnormal change in morphology or metabolic activity, be unable to transverse the female reproductive tract to the site of fertilization in sufficient numbers, or their ability to fertilize the oocyte or yield a developmentally competent embryo may be impaired.

Conventional *in vitro* evaluation of semen quality following the freeze thaw process, such as the assessment of concentration, motility and morphology are of limited value in assessing field fertility [4]. Various fluorescent staining techniques have also been used to evaluate sperm viability [5,6,7], capacitation status [8,9], membrane integrity [10], chromatin integrity [11], acrosome status [5,12] and mitochondrial activity [5,13], and while these measurements are useful for *in vitro* assessment of sperm they have limited ability to predict field fertility. The rate at which specific stains can be reduced by the mitochondria of sperm has also been used as an assessment of metabolic status [14]. It has been suggested that MTT (3- (4,5-Dimethylthiazol-2-yl) -2,5- diphenyl- tetrazolium bromide) may be used as a reliable indicator for bovine [14] and equine [15] sperm fertility. MTT is a yellow, water soluble tetrazolium salt which is reduced to water-insoluble purple formazan crystals in the mitochondria of living cells. The amount of formazan can be measured spectrophotometrically and therefore gives an estimate of the number of living cells in a sample. This method was first reported by Mosmann [16], who considered it to be a simple and inexpensive method to assess viability, and has been used widely on many cells types [14,17,18,19].

It has been reported that *in vivo* barriers to sperm transport can be mimicked *in vitro* using sperm migration tests as a tool to examine sperm quality [20,21]. These tests have been used for determination of sperm function in humans [22], goats [23] and bulls [24,25]. Recently, artificial mucus composed of hyaluronic acid [26] or polyacrylamide gel [25] has been used as a suitable and more consistent alternative to natural mucus. On the other hand other authors have suggested that there is no relationship between mucus penetration and field fertility [21,27,28].

Some authors have reported that *in vitro* fertilization (IVF) can be used as a tool to predict the field fertility of a bull or to discriminate among bulls of different field fertility in terms of both cleavage and blastocyst formation rates [29,30,31,32]. Others have reported a correlation between field fertility and cleavage rate alone [33,34] or blastocyst formation rate alone [35,36], while some authors have reported that IVF is not a useful predictor of field fertility [37,38,39].

The aim of the present study was to investigate whether differences in field fertility of bulls is reflected in differences (i) in the metabolic activity of sperm, in terms of their ability to penetrate mucus, their mitochondrial activity as well as their ability to survive the freeze-thaw process or (ii) in the ability to fertilize oocytes and produce viable embryos.

2. Materials and methods

2.1. Experimental design

All bulls used in this study were located in the National Cattle Breeding Centre (NCBC), Enfield, Co Meath, Ireland and were used for commercial AI in Ireland. Semen was diluted and then frozen in 0.25 mL straws, each containing approximately 80×10^6 spermatozoa per 1 mL. The study was carried out over two years and involved a separate sample of bulls each year. In Year 1, semen from six bulls was used of which three bulls (1 Holstein Friesian and 2 Limousin) were classified as having 'high fertility' and three Holstein Friesian bulls were classified as having 'low fertility'. Given the different breeds in the high fertility group, the experiment was repeated in Year 2 using semen from five Holstein Friesian bulls of which three were classified as having 'high fertility' and two were classified as having 'low fertility'. In Year 1 the field fertility of the 6 bulls was calculated as 90-day non-return rate (NRR; Table 1). Due to an updated record-