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Relationship between bovine fertility and the number of spermatozoa penetrating the cervical mucus within straws

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Abstract

In this study, by using a recently developed test technique, the relationship between the total spermatozoa number penetrating determined sites of bovine cervical mucus in straws and potential fertility of bulls, and other spermatological characteristics were investigated. Furthermore, we aimed to determine the effect on the test results, of two different incubation temperatures (37 and 41 °C) and two sperm penetration distance ranges (PDRs). Frozen semen samples of six Holstein bulls were used in the study. The bulls were divided into two fertility groups (high and low fertility) according to the "non-return rates" (NRR). For the penetration test, cervical mucus was drawn into transparent plastic straws and incubated with semen at 37 and 41 °C for 15 min. After the incubation, straws were frozen in liquid nitrogen vapour and stored at -20 °C. On the evaluation day, concentrations of spermatozoa penetrated to the PDRs, each of which was 2.5 mm, between 32.5 and 35 mm (first penetration distance range, PDR1), and 50 and 52.5 mm (second penetration distance range, PDR2) distance in the straws from the open end, were measured. When compared with the low fertility group, bulls from the high fertility group showed a higher number of spermatozoa at the determined PDRs, and a significant positive correlation was found between the total number of spermatozoa at the penetration distances and the NRR scores of the bulls.

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1. Introduction

The aim of the breeding industry is to identify genetically superior bulls and hence maximize the number of offspring produced by these selected bulls via artificial insemination. Fertility of selected bulls is important in achieving this aim. Fertility can be accurately evaluated by performing test inseminations in the field, but this method is expensive and time-consuming (Hallap et al., 2004), and allows a limited number of males to be tested. Conventional criteria, such as concentration, motility and morphology are of limited value for measuring the sperm fertilizing ability (Graham et al., 1980; Stålhammar et al., 1994; Hirano et al., 2001). Several assays have been developed to evaluate different sperm functional parameters such as ATP content in sperm, sperm penetration assay into oocytes, acrosomal membrane integrity along with acrosin activity and cervical mucus penetration (World Health Organization, 1992). Performing these functional tests is usually time-consuming and may require specialized equipment, limiting their clinical or field applications (Correa et al., 1997).

It has been reported that performing the cervical mucus penetration tests (CMPT) is more convenient compared to other sperm functional tests (Eggert-Kruse et al., 1989). In humans, a number of independent studies have shown that the fertilizing capacity of spermatozoa is strongly related to penetration of the cervical mucus (Alexander, 1981; Hull et al., 1984). Therefore, the use of CMPT in assessment of fertility in man has gradually become more generalized (Paul et al., 1985; Eggert-Kruse et al., 1989; Keel and Schalue, 2000). However, a limited number of studies have suggested no positive correlation between the migration capacity of spermatozoa and fertility in bulls (Matoušek et al., 1989; Galli et al., 1991; Verberckmoes et al., 2002). In most studies, the penetration distance of spermatozoa has undergone assessment (Okuda et al., 1988; Matoušek et al., 1989; Anilkumar and Devanathan, 1996) and it has been stated that assessment of penetration distances of spermatozoa in in vitro CMPT alone, may be insufficient for determination of fertility (Eggert-Kruse et al., 1989). It has been set forth that the disparity in results between different bovine studies and between bovine and human experiments can be explained by differences in the test methods (Verberckmoes et al., 2002).

Despite developments in the capillary tube method, the results can show variations according to laboratories and individuals, and that the method has some drawbacks, such as difficulty in standardization (Pandya et al., 1986). Recently, a new CMPT technique has been developed, in which transparent plastic straws are used instead of capillary glass tubes, and which depends on the determination of total number of spermatozoa penetrated to determined sites in bovine cervical mucus. This new modified CMPT (mCMPT) is said to be easily performed, does not require special equipment, reduces user mistakes and that it is very suitable for standardization (Taş, 2003).

The aim of the present study was to determine, by means of mCMPT, the relationship between the total number of spermatozoa penetrated to two different sites in bovine cervical mucus within the straws and the potential fertility of bulls. In addition, the effects on the mCMPT results of an incubation temperature (41 $^{\circ}$ C), which is higher than traditional incubation temperatures and the sperm penetration distance ranges (PDRs) to be tested were determined.

2. Materials and methods

2.1. Frozen bull semen

In the study, frozen semen of six Holstein bulls of the candidate bull status in a Progeny-Testing project, were used. Semen samples collected from bulls housed in the same raising and

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