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EXTENDED ABSTRACT

Proteomics to investigate fertility in bulls

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Abstract In dairy cattle breeding, herd reproductive management is the primary focus, affecting a large part of the general costs. A negative association was observed between the level of milk production and fertility. Some studies have shown that a significant percentage of reproductive failure is attributable to semen quality; therefore, if reproduction management is based on artificial insemination, then it is important to assess the fertility level of the sires. In this study, proteomic analysis was used to compare the protein expression profiles from sperm of high- and low-fertility bulls. Comparative proteomic analysis showed that expression of several proteins [nine different two-dimensional electrophoresis (2-DE) spots] is related to fertility level ($p \leq 0.05$). These proteins are involved in sperm-egg interactions and cell cycle regulation. Differences in protein expression levels might explain reductions in fertility due to mistakes in sperm-oocyte communication or in cell cycle regulation. Proteomics of sperm can be a valuable tool to identify protein expression changes related to fertility; in particular, 2-DE-based proteome analysis is very useful for the characterization of spermatozoa protein expression related to high- and low-fertility rates. Furthermore, analysis of expression profiles could be critical to the identification of protein biomarkers of bull fertility.

Keywords Proteomics · Fertility · Spermatozoa · Bovine

Abbreviations

ERCR	estimated relative conception rates
IPG	immobilized pH gradient
NRR	non-returns rate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
2-DE	two-dimensional electrophoresis
PCA	Principal component analysis

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Introduction

In dairy cattle bred at high production rates, herd reproductive management (cow replacement, veterinary costs for management of reproductive diseases, and cost of frozen semen) is the main task and affects a large part of the general costs. A negative association was observed between the level of milk production and fertility, which may be linked to genetic (pleiotropy and inbreeding) and physiological factors (metabolic diseases) (Bach et al. 2008). While much progress has been made in cattle management (facilities, nutrition, and hygiene) to increase female fertility, studies are still lacking to increase bull reproductive efficiency. Some studies have shown that a significant percentage of reproductive failure is attributable to semen quality and not to cow problems (DeJarnette et al. 2004); therefore, reproduction management based on artificial insemination (AI) is important for the assessment of the fertility level of sires. Currently, the method most used to measure fertility is the NRR, the percentage of cows without heat signs at a given number of days from AI with respect to the total number of cows inseminated (Koops et al. 1995; Liu et al. 2008). There is an association between the number of vital sperm and fertility until a threshold is reached, beyond which fertility does not increase (DeJarnette et al. 2004). The threshold value varies from bull to bull and represents the fertility level of the sire.

Materials and methods

Six bulls (T1-T6) were selected for high fertility and low fertility levels according to the values of ERCR obtained from the Holstein National Breeders Association (ANAFI). ERCR were evaluated from the percentage of non-returns at 56 days, adjusted for environmental factors (herd, month of insemination, age of cow, days in milk, milk production, etc.). The ERCR value for each bull can be interpreted as the NRR, corresponding to inseminations by that bull relative to all other bulls that were used in the same herd.

The ERCR data set was analyzed to identify two groups of bulls with high and low fertility levels, with an average ERCR reliability equal to 96.7% (range from 92% to 99%). The six bulls had the following values of ERCR: T1 low fertility (−4.74), T2 low fertility

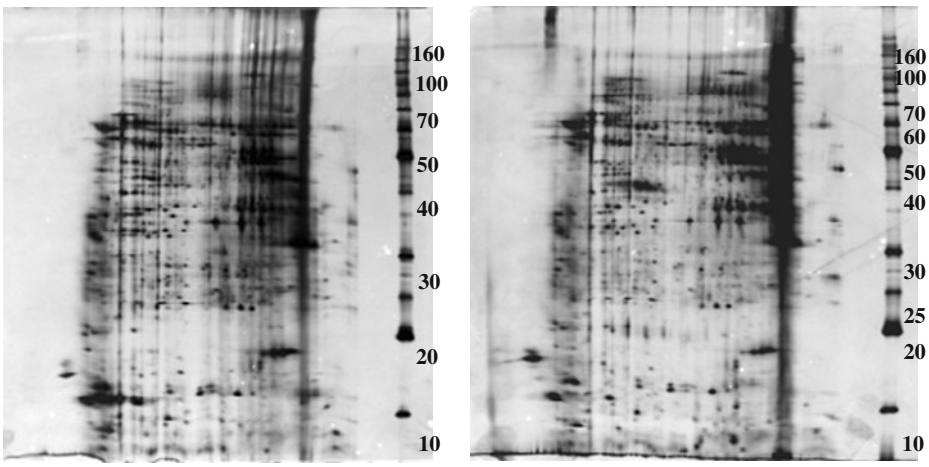

Fig. 1 Representative 2-DE maps of sperm from low-fertility bulls (left) and from high-fertility bulls (right)

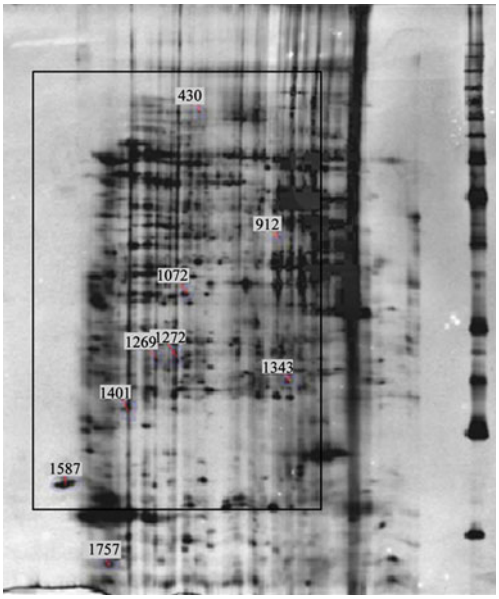

Fig. 2 Proteins differentially expressed in high- and low-fertility bulls. Numbers has been assigned directly by image analysis software; they indicate proteins resulted significantly different by PCA analysis

(−3.93), T3 low fertility (−3.52), T4 high fertility (+2, 39), T5 high fertility (+2.26), and T6 high fertility (+2.62). Commercial frozen semen samples were thawed in a 37°C water bath for 2 min and then centrifuged at 10,000 × *g* for 10 min to remove the thawing solution. After centrifugation, spermatozoa pellets were re-suspended in 200 μL solution containing 7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 15 mM Tris, and 2% ampholine pH 3.5–10 and sonicated for 30 seconds.

The first dimension was performed using IPG strips, pH 3–10, NL 13 cm (GE Healthcare) and the second dimension was by SDS-PAGE on 12% polyacrylamide gels. Gels were stained using a standard silver nitrate staining protocol. Image analysis was

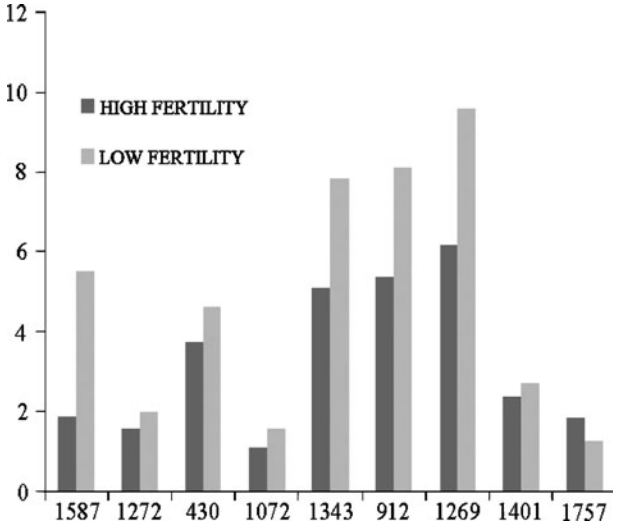

Fig. 3 Changes in the levels of protein expression in high- and low-fertility bulls ($p<0,05$; Student's I-test and one-way ANOVA)

performed using Progenesis SameSpots (Nonlinear Dynamics) software. Student's *t*-test and one-way ANOVA were used to confirm the level of significance between different groups: p values less than or equal to 0.05 were considered statistically significant.

Results

Representative 2D PAGE maps related to high and low fertility conditions are shown in Fig. 1. Principal component analysis (PCA) was used to analyze 2-DE data of six sperm samples. PCA analysis confirmed ERCR data, and PCA classification was the same as ERCR classification. PCA results showed nine different 2-D spots related to high and low fertility bulls (Fig. 2). The proteins highlighted in the box of Fig. 2 show an increase in expression in bulls with low fertility compared to high fertility bulls. The protein expression level represented by spot 1757 is higher in the high-fertility bulls. The quantitative differences in protein expression of nine different 2-DE spots showed that their expression levels were related to high and low fertility ($p \leq 0.05$) as shown in Fig. 3.

Discussion

Comparative proteomic analysis showed that the expression levels of several proteins are related to high and low fertility. These proteins are involved in sperm-egg interaction and cell cycle regulation. Differences in expression levels of these proteins might explain the reduction in fertility due to mistakes in sperm-oocyte communication or in cell cycle regulation (Peddinti et al. 2008; de Mateo et al. 2007). Proteomics analysis of sperm can be a valuable tool to identify protein changes related to fertility, and 2-DE-based analysis is a powerful tool to characterize the expression levels of spermatozoa proteins related to high and low fertility rates. Furthermore, analysis of expression profiles could help identify protein biomarkers of bull fertility.

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