Relation between bull sperm respiratory burst activity and the in vitro fertilization rate: a new approach to evaluate bull´s fertility

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Introduction

Several techniques have been employed to determine the semen ability to fertilize oocytes, as it is generally accepted that there is a connection between the fertility of semen and its measurable properties. Concerning sperm metabolism, abundant literature has been published on the metabolic behaviour of spermatozoa of several species. Although hydrogen peroxide (H_2O_2) is the major reactive oxygen species (ROS) produced by sperm, no study has been published relating the burst activity of sperm and their ability to fertilize. Spermatozoa, like all cells living under aerobic conditions, constantly face the oxygen paradox: oxygen is required for life, but the oxidative metabolism of biological molecules can be toxic due to the formation of highly reactive oxygen species that can modify cell functions and their viability. Particularly in sperm, high concentration of H_2O_2 is known to induce nuclear DNA fragmentation and lipid peroxidation resulting in cell death.

Objectives

The objective of the present study is to establish the relationship between the burst activity of frozen/thawed bovine spermatozoa and its ability to fertilize in vitro.

Materials and Methods

- *Animals*
  - Three straws per bull of different ejaculates of a total of 8 bulls (Holstein Frisian) were used in this study. After thawing the straw content was split in two identical parts. One was used for in vitro fertilization, while the other one was used for flow cytometry to evaluate sperm viability and the sperm oxidative burst activity.
- *In vitro embryo production*
  - Bovine ovaries collected at a local slaughterhouse
  - Cumulus oocytes complexes (COC's) (n=3250) were aspirated and cultivated (24 h , 38,5 ºC, 5% CO_2) in M199 medium
  - Frozen/thawed sperm selected by "Swim Up method"
  - After maturation the oocytes were rinse twice in fertilization media (Fert-TALP) and matured with 1 x 10^6 spz/ml (38.5ºC, 5% CO_2)
  - Cleavage and development of embryos to the blastocyst stage were assessed at 48 and 216 h post insemination.
- *Measurement of sperm respiratory burst activity*
  - Evaluated by flow cytometry, measuring the intracellular oxidation 2`,7`-dichlorofluorescein diacetate (DCFH) to 2`,7` dichlorofluorescein (DCF) by H_2O_2-production. The mean fluorescence intensity of the analyzed sperm cells (n = 15 000) was determined after gating the cell population by forward and side light scatter signals (FSC and SSC).

Results

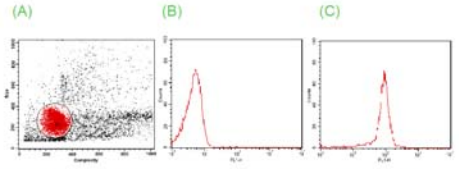

Figure 1 - Typical results generated by flow cytometry evaluating the sperm burst activity, indirectly by the fluorescence of DCF. In (A) each dot represents a single cell; its position indicates its forward scatter (FSC) intensity value (cell size), and its side scatter (SSC) intensity value (cell granularity). The region indicated as R1 represents the population of the analyzed sperm cells. Both histograms indicate the number of sperm cells (counts) representing green fluorescence (FL1-H): (B) represents the bull with lowest sperm burst activity while (C) represents the bull with highest sperm burst activity.

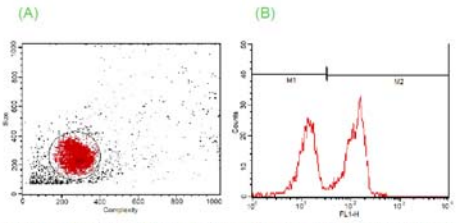

Figure 2 -Typical results of sperm's oxidative burst with two distinct populations in the same straw. Besides as evaluated by means of FSC and SS the sperm population seem to be homogeneous (A), two distinct populations of burst activity are identified (B). One with low (M1) and the other with high fluorescence intensity (M2).

The correlation between burst activity and fertilization rate and further embryo development to blastocysts was respectively 95.6% and 87.8% ($p \leq 0.001$).

Conclusions

- Bulls in which the burst activity was higher resulted in better results for in vitro fertilization and further embryo production
- Generation of H_2O_2 is not merely a means of discarding toxic waste products, nut instead that it plays a significant role in sperm metabolism
- Measurement of intracellular H_2O_2 concentration can thus be a valuable tool to predict the sperm fertilization capacity: sperm cells with high H2O2 concentration have high metabolic activities and consequently higher fertilizing capacities
- The sperm oxidative burst activity, as measured by flow cytometry, can thus be an excellent method to predict the potential fertility of different bulls.

Acknowledgements

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