Boar Sperm Proteins

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Artificial insemination with extended liquid boar semen is widely used in the swine industry. The identification of the relationship between boar sperm characteristics and fertility could be of substantial importance to reproduction management.

Keywords: artificial insemination ; boar ; field fertility ; proteins

1. Introduction

Boars play a key role in pig farms' productivity and economic efficacy. In the swine industry, the fertilization of sows is exclusively achieved by artificial insemination (AI) with extended liquid boar semen. This choice has contributed to faster genetic improvement and reduced production costs, while the boars' impact on the reproductive outcome has substantially increased [1]. Numerous researchers have attempted to correlate the qualitative sperm characteristics with fertility, deriving conflicting results. Initial difficulties were due to a lack of objectivity and repeatability, attributable to the use of subjective sperm assessment techniques [2]. However, the development of computer assisted sperm analyzing systems (CASA) reduced the influence of human factors, thus improving the accuracy and objectivity of the measurements [3].

Today, the farrowing rates by artificial insemination exceed 90%, and the number of live-born piglets is higher than 12. The achieved litter size of 12–14 piglets increases the reproductivity and reduces the early culling of the sows, improving the income of the pig farms [4].

Obviously, fertility is a complex process, influenced by both boar and other factors regarding animal management. The cellular factors associated with the fertilization failure of spermatozoa are not always clearly explained, with sperm and seminal plasma proteins needing to be involved in research studies to clarify this issue. Reactive oxygen species (ROS) are beneficial for sperm hyperactivation and acrosome reaction [5]. Nonetheless, the imbalance between ROS and the antioxidant mechanisms, which leads to oxidative stress, can be destructive, causing cell death and reducing fertilizing capacity [6]. Glutathione peroxidase (GPx) regulates small changes in the concentration of H2O2 or other derivatives. Alvarez and Storey [7] highlighted its protective role against the loss of sperm motility due to peroxidation of membrane lipids. In humans, non-expression of GPx in sperm causes infertility [8]. The family of the glutathione peroxidase is categorized in five classes (GPX1-GPX5), based on their sequence and location. GPX5 is a specialized protein, which inhibits the early acrosome's response when the spermatozoa are located in the epididymis' tail. Moreover, Kilian et al. [9] identified one sperm plasma protein of 55 kDa molecular weight which is positively related with bull fertility. This protein was later identified as osteopontin (OPN) [10]. It is believed that OPN attaches to spermatozoa during ejaculation, and it remains attached until they reach the isthmus of the fallopian tubes [11]. In camels, OPN has been positively correlated with fertility [12]. Additionally, it has been reported to improve the effectiveness of swine [13], buffalo [14] and cattle [15] in vitro fertilization (IVF). In boar spermatozoa, the Western Blot method identified at least two forms of OPN with different molecular weights, i.e., different isoform versions, possibly with different functions [16]. Furthermore, the body responds to heat stress by increasing the synthesis of a group of proteins called Heat Shock Proteins (HSPs) [17]. This group of proteins makes sperm more resistant to temperature changes. The chaperone HSP90 is the most studied Heat Shock Protein [18] which increases sperm thermal resistance and protects spermatozoa from apoptosis and oxidative stress [19] [20]. It has been found that lower expression of HSP90 is related to a greater cold stress sensitivity of sperm [21] and to a subsequent reduction of motility [22].

2. Animals and Semen Collection

Eighteen (18) crossbred boars, kept in a farrow-to-finish farm under veterinary monitoring, were used in this study. All animals involved were vaccinated against major swine pathogens and dewormed according to the farm's regular preventive scheme. In total, 65 ejaculations (3–4 per boar) were collected, processed, and then used to inseminate 468 sows of parity ≥ 2 with conventional AI throughout a year. From each ejaculate, an average of 7.2 sows were inseminated.

For each ejaculate, the fertility outcomes (live-born piglets, proportion of litter sizes with ≥12 piglets and farrowing rate) derived from its use were recorded. The average values were then calculated and used for further analysis.

The boars and sows were housed under intensive farming conditions, where a balanced diet and ad libitum water were provided [23]. The boars were housed in individual pens under controlled environmental conditions. The whole ejaculate was collected with the “gloved hand technique”. The gelatinous phase of each ejaculate was removed after filtration and discarded. All collected samples fulfilled the quality criteria for AI semen dose preparation (at least 300 × 10^6 sperm/mL, 70% motile spermatozoa, and 80% spermatozoa with normal morphology).

After that, the semen was divided into two aliquots, and different processes were followed according to the respective laboratory tests. The first aliquot was processed for proteomic analysis, and the second one was used for the assessment of sperm quality and functionality variables.

3. Preparation of Sperm and Seminal Plasma Proteins for Proteomic Analysis

In the beginning, the first portion was mixed with a protease inhibitor cocktail (4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatin A, E-64, leupeptin, bestatin, aprotinin) and centrifuged (640× *g*; 15 min; 17 °C) to separate sperm cells and seminal plasma [24] (González-Cadavid et al., 2014). Seminal plasma was centrifuged for a second time (10,000× *g*; 15 min; 17 °C), and finally the supernatant was stored at −80 °C until further examination.

The sperm pellet was diluted with Phosphate Buffer Solution (PBS) to a concentration of 1.5 × 10^9 spermatozoa/mL. The diluted sperm was firstly centrifuged (640× *g*; 3 min; 17 °C), then washed with 10 mL PBS and finally re-centrifuged (800× *g*; 5 min; 17 °C) [25]. The last sperm pellet produced was resuspended with HAM F-10 1X (Thermofisher® Scientific, Regensburg, Germany), and the sample was once again centrifuged, whereas the supernatant was removed. Following this, the spermatozoa were solubilized in NP-40 lysis buffer (50 mM Tris–HCL pH 7.4, 250 mM NaCl, 5 mM EDTA, 1% Glycerol, 0.5% NP-40, 1 mM DTT, 1 mM PMSF 100 mM, 1 × protease inhibitor cocktail (Roche, Athens, Greece)) and the aliquot was stored at −80 °C until further analysis.

4. Semen Sample Processing for the Performance of Sperm Analysis

The second aliquot of collected semen was extended with a commercial extender (M III®, Minitube, Tiefenbach, Germany) to a final concentration of 30 × 10^6 spermatozoa/mL, divided to insemination doses and stored in the farm’s storage facilities at 17 °C. One semen dose per boar was used, transported to the laboratory (within an hour) inside an air-conditioned isothermal box (Minitube, Tiefenbach, Germany) adjusted to 17 °C for further analysis.

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