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# Nanotechnology-based selection of boar spermatozoa: growth development and health assessments of produced offspring

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ABSTRACT

The heterogeneous population of spermatozoa within the semen ejaculate influences the sire fertility. The current design of magnetic nanoparticle conjugates allows for selective targeting and removal of non-viable spermatozoa within the semen ejaculate. However, the safe application of this process, termed as nanoselection or nanopurification in previous studies, in food animal production and toxicity concerns has yet to be explored. Here, we assessed the fertility potential of nanoselected boar spermatozoa and the subsequent post-natal growth and health characteristics of resulting offspring. Semen doses were harvested from three fertile boars (n = 4 doses per boar) and split in two groups (2 doses/boar). Six semen doses (2 per boar)) were subjected to the targeted depletion of acrosome membrane damaged and apoptotic spermatozoa (nanoselected). Meanwhile, the remaining semen doses (n = 6) were maintained in the shipping Styrofoam box (control). The motility characteristics of both control and nanoselected spermatozoa were evaluated before and after nanoselection, followed by their use for double inseminations of six estrus synchronized gilts (2 doses/boar/gilt; 3 gilts /control or nanoselected). In comparison to the controls, the computer-assisted sperm analyzer (Hamilton-Thorne) revealed greater motion characteristics of nanoselected spermatozoa, with a significantly higher proportion of progressive spermatozoa and straightness ($P < 0.05$). The fertility potential of nanoselected spermatozoa was not compromised, and produced offspring showed growth rates and weight gains at market that were comparable to their counterparts born from control spermatozoa ($P > 0.05$). Various developmental and health parameters of produced offspring such as hepatic cytochrome P450 enzyme activities, blood glucose and immunoglobulin G concentrations, hematocrit, and white blood cell proportions were similar between and across all pigs (control and nanoselected). In addition, reproductive tracts of females born from nanoselected spermatozoa showed no indication of impaired fertility potential, although a significant shortened uterine horn length was measured (56.3 ± 2.6 cm vs. 64.4 ± 2.2 cm in the control group, P = 0.04). In conclusion, findings revealed no obvious perturbations of sperm function following nanoselection, while post-natal growth, development, or health data of derived offspring suggest absence of inflicted sub-lethal toxicities attributable to sperm nanoselection. This study supports the safe use of the proposed nanotechnology-based selection for effective semen handling in terminal line swine production systems.

## 1. Introduction

Pork has become one of the most widely consumed meats in the world (McLeod, 2011), obliging sustainable production in swine farms that relies mainly on the maintenance of high genetic and reproductive merit animals. Advancements in breeding techniques through the use of artificial insemination have enhanced opportunities for producers to increase the genetic quality of offspring. However, advanced livestock breeding techniques have yet to successfully address male in/sub-fertility conditions associated with disproportionate populations of viable and non-viable spermatozoa within ejaculates (Roca et al., 2016).

Various intrinsic (i.e., genetic, health) and/or extrinsic (i.e., hot stress, seasonal variations) factors influence the occurrence and extent of sperm damages, while existing routine techniques of semen quality

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evaluation do not permit the elimination of damaged spermatozoa from ejaculates (Sutovsky, 2015). These techniques are mainly informative and useless, as semen ejaculates not meeting quality standard are still rejected, causing substantial losses in commercial boar studs (Knox et al., 2008). Damaged spermatozoa form aggregates, which likely impede with viable sperm displacement during migration through the female reproductive tract (Saleh et al., 2003), prompting for the need to develop new methods to extract damaged spermatozoa from semen ejaculates.

Nanobiotechnology is a novel and growing field of science dealing with nano-size scaled particles (nanoparticles) of one to 100 nm in diameter, having potential to fulfil current demands for advanced assisted reproduction in swine. Synthesized nanoparticles have shown multifunctional applications in biomedical research, with magnetic nanoparticles becoming of particular interest in reproductive biology due to their controllable size production, cell-specific targeting mechanisms, and magnetic non-invasive characteristics (Sutovsky and Kennedy, 2013; Barkalina et al., 2014; Jha et al., 2014; Feugang, 2017). Magnetic tools (MNP and magnetic assisted cell-sorting or MACS) have been applied as a labeling and sorting technique for mature spermatozoa (Ben-David Makhluf et al., 2006; Gil et al., 2013; Feugang et al., 2015; Valcarce et al., 2016; Vasquez et al., 2016), while the use of MNP conjugates for targeting and removal of damaged spermatozoa has allowed harmless selection of boar and bull semen (Odhiambo et al., 2014; Feugang et al., 2015). This selection procedure, termed as nanoselection, enriches semen doses with desired viable spermatozoa leading to successful pregnancies (Odhiambo et al., 2014; Feugang et al., 2015; Durfey et al., 2017), and has potential to become a promising routine method to rescue viable spermatozoa in poor quality semen doses (Barkalina et al., 2016). However, the bio-application of nanoparticles has triggered concerns for potential genotoxic and cytotoxic, which long-term affects during post-natal development of offspring remain unclear.

Indeed, the small size and large surface-to-volume ratio of nanoparticles allow for enhanced reactivity and efficient cell targeting that increases the risk of their diffusion through cellular membranes and tissue barriers to initiate cytotoxicity (Brunner et al., 2006; Singh et al., 2010). Induced-cytotoxicity by MNP or their aggregates could lead to harsh impairments of critical components in cellular function, resulting in membrane damage, formation of apoptotic bodies, or impairments in mitochondrial function (Jeng and Swanson, 2006; Singh et al., 2010). The occurrence of such damages in spermatozoa may jeopardize their viability and fertility potential. Although available data show enhanced sperm functionality following nanoselection through MNP (Ben-David Makhluf et al., 2006; Odhiambo et al., 2014; Feugang et al., 2015; Durfey et al., 2017), the likelihood of sub-lethal damages induced during this process having mid- or long-term effects during *in utero* and post-natal offspring development should not be ruled out.

From this background, it appears that the current knowledge of the effects of sperm nanoselection on generated offspring is still limited or unknown. In the proposed study, the motion characteristics of spermatozoa were analyzed post-nanoselection and post-weaning (to market) evaluation and comparison of growth (weight and female reproductive tract morphometry) and health (glucose, IgG, white blood cell composition, hematocrit, and hepatic cytochrome P450 activity) parameters pig offspring born from control and nanoselected spermatozoa were performed.

## 2. Materials and methods

Otherwise indicated, all reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA. All animal care and use were performed according to protocols approved by the Institutional Animal Care and Use Committee of Mississippi State University.

### 2.1. Magnetic nanoparticle synthesis

Iron oxide ($Fe_3O_4$) magnetic nanoparticles (MNP) were coated with lectins and annexin V to selectively bind acrosome reacted and apoptotic (damaged) spermatozoa. Functionalized magnetic nanoparticles were synthesized under an intellectual property (Clemente Associates Inc.; Madison, CT, USA) and used for sperm labeling (Feugang et al., 2015; Durfey et al., 2017).

### 2.2. Sperm labeling and nanoselection

Freshly harvested semen of three sexually mature boars were mixed with NUTRIXcell extender (IMV Technologies, Maple Grove, MN, USA) and four insemination doses (approximately $3\times10^9$ spermatozoa /80 ml in a plastic bag) were prepared for each boar at a commercial stud (Prestage Farms, West Point, MS, USA). A total of twelve doses harvested from three fertile boars (n = 4 doses /boar) were split in six doses (n = 2 /boar). A group of six doses was mixed with 0.3 mg of annexin V- conjugated MNP, to target apoptotic spermatozoa and then with 0.3 mg of lectin-conjugated MNP, to target acrosome reacted spermatozoa in two successive nanoselection processes, as previously described (Durfey et al., 2017). Calculations were made to target approximately $0.6\times 10^9$ moribund spermatozoa, corresponding to $2\times 10^9$ sperm /mg of each MNP conjugate. Following MNP exposure (30 − 45 min), mixtures were placed against an external magnetic field for 10 min to trap nanoparticle-bound spermatozoa (non-viable). This procedure, or nanoselection, took place at room temperature and nanoselected (viable) spermatozoa were eluted in new bottles. The other half of semen doses (n = 6, of 2 doses /boar) were maintained in their original insemination bags, kept in the Styrofoam box (16–18 °C) and served as controls for analyses and inseminations.

### 2.3. Analysis of sperm motility and motion characteristics

Aliquots of control and nanoselected spermatozoa were analyzed with a Computer-Assisted Sperm Analyzer (CASA: HTM-IVOS; Hamilton-Thorne Biosciences; Beverly, MA, USA), as previously reported (Feugang et al., 2015; Durfey et al., 2017). Briefly, spermatozoa were analyzed on a 20 μm Leja® Count-4-chamber slides (Nieuw Vennep, The Netherlands) using pre-set values of CASA. Sperm motility (percent of total, progressive, rapid - ≥ 30 μm/sec-, and static) and motion parameters [average path (VAP), straight line (VSL), and curvilinear (VCL) velocities in μm/s; lateral head aptitude (ALH, in μm), beat cross frequency (BCF, in Hz), straightness (STR or VSL/VAP, in %), and linearity (LIN or VSL/VCL, in %)] were evaluated.

### 2.4. Animals and artificial insemination

Both semen groups were used for immediate intra-cervical inseminations of six sexual mature gilts (Yorkshire x Duroc - Prestage Farms; West Point, MS, USA). Each gilt was bred with either two control or two nanoselected semen doses of the same boar, with three gilts bred for each semen group. Breeding consisted of a double insemination, as previously described (Feugang et al., 2015), and all pigs were confirmed pregnant by ultrasonography (approximately 30 days post-insemination). Thirty five (35) and 38 viable offspring were weaned from gilts bred with control and nanoselected spermatozoa, respectively. Thereafter, ten offspring of equal genders (5 females and 5 males) born from each control (n = 10) and nanoselected (n = 10) spermatozoa were randomly selected and grown to market size. All selected offspring (n = 20) were administered iron (intramuscular) at 3 days of age and males were castrated at approximately two weeks of age. All offspring were dewormed (Ivomec – Merial; Duluth, GA, USA) and vaccinated at weaning (28 days of age). The growing pigs (n = 20) were monthly weighted until harvest at approximately 165 days of age, corresponding to the market size of ≈ 132 kg. All animals were maintained under a