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Successful laparoscopic insemination with a very low number of flow cytometrically sorted boar sperm in field conditions

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ABSTRACT

The aim of this study was to develop a useful procedure for laparoscopic insemination (LI) with sex-sorted boar spermatozoa that yields adequate fertility results in farm conditions. In experiment 1, we evaluated the effects of single (oviducts) and double (oviducts and tips of the uterine horns) LI with X-sorted sperm on the reproductive performance of sows. Sows (N = 109) were inseminated once as follows: (1) single LI with 0.5×10^6 unsorted sperm per oviduct; (2) single LI with 0.5×10^6 sex-sorted sperm per oviduct; or (3) double LI with 0.5×10^6 sex-sorted sperm per oviduct and 0.5×10^6 sex-sorted sperm per uterine horn. The farrowing rates were lower ($P < 0.05$) in sows inseminated with sex-sorted sperm (43.2% and 61.9% for the single and double insemination groups, respectively) than in sows from the unsorted group (91.3%). Within the sex-sorted groups, the farrowing rate tended ($P = 0.09$) to be greater in sows inseminated using double LI. There were no differences in the litter size among groups. In experiment 2, we evaluated the effect of the number of sex-sorted sperm on the reproductive performance of sows when using double LI. Sows (N = 109) were inseminated with sex-sorted sperm once using double LI with: (1) 0.5×10^6 sperm per oviduct and 1×10^6 sperm per uterine horn; or (2) 1×10^6 sperm per oviduct and 2×10^6 sperm per uterine horn. Similarly high pregnancy (90%) and farrowing (80%) rates were achieved in both groups. The sows inseminated with the highest number of sperm tended ($P = 0.09$) to have more piglets (10.8 ± 0.7 vs. 9.2 ± 0.6). A high female proportion (number of female births divided by the total of all births $\geq$0.92) was obtained in both experiments using X-sorted sperm. Our results indicate that the double LI procedure, using between 3 and 6×10^6 sex-sorted sperm per sow produces adequate fertility at the farm level, making sperm-sexing technology potentially applicable in elite breeding units.

1. Introduction

The practical use of sexed semen could have a significant effect on swine production, because it allows planned matings for a specific sex of piglet [1]. However, the commercial use of sexed semen in porcines is limited, primarily because the current outputs of sorting machines (28×10^6 sperm for each sex per hour) [2] are insufficient for conventional artificial insemination, which usually requires 2 to 3×10^9 sperm per insemination [3,4]. In recent years, a new procedure for deep uterine insemination (DUI) using a low number of sperm has been developed, and this technology is now commercially available [5,6]. This procedure produced optimal farrowing rates and litter sizes for inseminations using unsorted sperm counts as low as 150×10^6 [7]. When DUI was performed with 50 to 140×10^6 sex-sorted sperm

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per insemination, acceptable farrowing rates and litter sizes were achieved [8–10], however, these rates were still far from those necessary for commercial use. These results indicate that, even when DUI technology is used, the number of sex-sorted sperm required to obtain high fertility is still too high. Although this drawback could be overcome if multisorter facilities were available, new procedures for insemination with a very low number of sex-sorted sperm are currently needed. When deeper insemination is performed, the number of inseminated spermatozoa necessary to obtain high fertility can be decreased (reviewed in [11]). A number as low as 10×10^6 unsorted sperm per sow can be used without compromising fertility when the sperm are deposited using surgical laparotomy next to the uterotubal junction (UTJ) [12,13]. However, despite the usefulness of the laparotomy procedure, the postoperative distress and the possibility of complications preclude the practical application of this procedure at the farm level [14]. The laparoscopic insemination (LI) technique is less invasive than laparotomy and can be performed on farms by technically trained personnel [15]. This procedure has been described as a useful alternative for insemination with a very low number of spermatozoa in swine (reviewed in [16,17]). In agreement with these findings, satisfactory fertilization rates were achieved using LI with 5 to 10×10^6 unsorted sperm in the tip of each uterine horn [18] or with 0.3×10^6 sex-sorted sperm in the oviductal ampulla [19]. However, the embryos were collected 1 to 5 days after LI in these studies, and the sows were not allowed to farrow; therefore, no data are available on the reproductive performance of sows when LI is performed with a very low number of sex-sorted sperm. In addition, important aspects of this procedure remain undefined.

The objective of the present study was to develop a successful procedure for LI with sex-sorted boar spermatozoa. For this purpose, we evaluated the effects of the site of sperm deposition and the number of sperm used for insemination on the reproductive performance of sows in farm conditions.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich Co. (Alcobendas, Madrid, Spain) unless otherwise stated.

All experimental procedures used in the present study were carried out in accordance with the 2010/63/EU EEC Directive for animal experiments and were reviewed and approved by the Ethical Committee for Experimentation with Animals of the University of Murcia, Spain.

2.1. Animals

Three mature Pietraine boars from the breeding center of AIM Ibérica (Calasparra, Murcia, Spain) were used in this study. The animals were housed in individual crates at a controlled temperature. Ejaculates were collected twice per week using the regular semen collection protocol for commercial AI.

Multiparous (3-6 parity) crossbred sows (Landrace × Large-White) from a commercial farm (Agropor S.L., Murcia, Spain) were randomly selected on the day of weaning and allocated to individual crates in a mechanically ventilated confinement facility. Food was restricted for 24 hours before the laparoscopic procedures.

2.2. Semen collection and spermatozoa preparation for sex sorting

Sperm-rich fractions were collected using the gloved hand method, diluted (1:1) in prewarmed (35 °C) Beltsville Thawing Solution (BTS) [20] and transported to the laboratory at 20 °C to 22 °C within 2 hours of collection. The semen samples were evaluated using standard laboratory techniques, and only ejaculates that fulfilled the following requirements were used: >80% motility, <10% acrosome abnormalities, and <15% abnormal sperm morphology. Samples were rediluted in BTS to a concentration of 100×10^6 sperm per mL and processed for sorting as previously described [21]. Briefly, 5 μL of Hoechst 33342 solution (5 mg/mL stock solution) were added to each 1-mL aliquot containing 100×10^6 spermatozoa. After the spermatozoa samples had been incubated in the dark at 35 °C for 50 minutes and filtered through a 30-μm nylon mesh filter, 1 μL of food dye (0.002% wt/vol; FD&C #40; Warner Jenkinson Company Inc., St. Louis, MO, USA) was added to each sample; the sample was then passed through the flow cytometer.

2.3. Flow cytometric sex sorting of sperm

X-chromosome-bearing sperm were separated using the Beltsville Sperm Sorting Technology [22] with a high-speed sorting system (SX MoFlo; Dako Cytomation Inc., Fort Collins, CO, USA) operated at 40 psi and equipped with a UV laser set to 175 mW output (Spectra Physics 1330, Mountain View, CA, USA). The samples were sorted using a sheath fluid described by Del Olmo et al. [23]. The samples were collected in 50-mL tubes that had been precoated with 1% BSA (wt/vol) in PBS. These tubes contained 2.5 mL of TES-Tris-glucose supplemented with 2% egg yolk as the collection medium [23]. A total of 20×10^6 sorted spermatozoa were collected per tube in an approximate volume of 25 mL (sorting rate: $18–20 \times 10^6$ sperm per hour). After sorting, the samples were pooled and processed for insemination. The pools of sorted sperm were centrifuged at $3000 \times g$ for 4 minutes at 21 °C. The supernatant was discarded and the pellets were re-extended using BTS to concentrations of 5 or 10×10^6 sperm/mL depending on the experiment. The samples were evaluated after concentration adjustment. Sperm motility was evaluated using a computer-assisted semen motility analysis system (ISAS, Proiser SL, Valencia, Spain) following the procedure described by Cremades et al. [24]. Sperm viability was assessed using a BD FACSCanto II flow cytometer (Becton Dickinson and Company, Franklin Lakes, NJ, USA) following the procedure described by Martinez-Alborcia et al. [25]. After evaluation, the samples were stored at 22 °C for a maximum of 12 hours until the time of insemination.

2.4. Management of sows

2.4.1. Synchronization and superovulation treatments

Superovulation was induced by injecting each female intramuscularly with 1250 IU of equine chorionic