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Optimization of post-cervical artificial insemination in gilts: Effect of cervical relaxation procedures and catheter type

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ABSTRACT

Post-cervical (pC) artificial insemination (AI) has been successfully developed for application in multiparous sows, although it has proved problematic in gilts. This study analyzes the use of pC-AI in gilts by two experiments. In the first experiment, the efficiency of pC-AI in gilts was evaluated using a multi-ring multiparous catheter (MpC), which led to 23.1% of the gilts being successfully inseminated. In gilts where insemination was not possible using an MpC, two alternatives were applied before a second attempt at insemination: 1) Vetrabutin Chlorhydrate (VC) was intramuscularly injected in order to relax the cervix; or 2) Warm extender (WE) was deposited in the cervix to modify the cervical muscle dynamics. After the application of these treatments, the success rates achieved with the MpC were 34.2% and 23.8% for VC and WE, respectively. No statistically significant differences were found in the reproductive parameters measured [farrowing (%), litter size and fecundity index] between the use of MpC, or the MpC combined with VC or WE, compared with gilts inseminated by cervical AI (control group). In the second experiment, new catheters based on the anatomical characteristics of gilts (GpC) were used, and the rate of successful pC-AI application were compared (experiment 2a): a) MpC: control; b) GpC1: multi-ring catheter of Ø 16 mm and inner cannula of Ø 3.5 mm; c) GpC2: a multi-ring catheter of Ø with an inner cannula of Ø 2.5 mm. The highest rate of successful cannula penetration was reached in the GpC2 group (60.3%) followed by GpC1 (37.0%) and MpC (19.6%) ($p < 0.05$). There were no differences in the above mentioned reproductive parameters using the three catheters compared with cervical AI method (control group). Moreover, prior cervical AI did not improve subsequent pC-AI application 24 h later (experiment 2b). In conclusion, Vetrabutin Chlorhydrate, warm extender or the new catheters can be considered as useful tools for improving the success rate of pC-AI technique in gilts.

1. Introduction

The main goal of mating or artificial insemination (AI) is to ensure that an adequate population of spermatozoa reaches the site of fertilization during the peri-ovulatory period. Cervical semen deposition is still widely used on farms for AI, whereby female pigs are inseminated with 2 to 3 billion sperm cells in an 80 to 100 mL volume. Over the years different approaches have been tried to improve cervical AI (C-AI) efficiency. For example the addition of substances such as oxytocin to the insemination doses [1,2] or the injection of substances such as PGF2α directly into the vulva before C-AI [1,3]. These methodologies reduce the problem of seasonality in reproduction, increasing conception rates and litter size.

In the past two decades, new strategies have been developed aimed at depositing the semen closer to the site of fertilization using a lower volume and number of cells than in C-AI (reviewed by

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[4]). Such methods avoid the transit of spermatozoa through part of the female tract, ensuring that an optimal functional sperm population reaches the oviduct at the time of ovulation. In general, the pig AI industry aims to optimize the boar ejaculate by decreasing the number of spermatozoa inseminated per dose, while maintaining the same efficiency in terms of pregnancy rate and litter size afforded by traditional insemination (C-AI) (reviewed by [5]).

One such technique is known as post-cervical artificial insemination (pC-AI) or intrauterine insemination [6–10], which involves depositing the sperm in the uterine body, after the cervix and just before the uterine bifurcation. pC-AI has been applied in the field with very encouraging results, reducing the number of sperm used to 1×10^9 (3-fold decrease) while maintaining the fertilization results obtained with cervical deposition [6,10].

Most studies of pC-AI have been carried out in multiparous sows [6,8–10], while fewer studies refer to primiparous sows [7,11–13] and hardly any to gilts [14,15]. The difficulty involved in inserting the insemination cannula could be due to several factors, one of which might be the size of the uterus. It is known that the reproductive tract of gilts is smaller than that of sows, which means that new insemination techniques or catheters specifically adapted to gilts are necessary.

In swine husbandry, sow replacement in a breeding farm is estimated at 20 to 50% of the total number of sows annually [16,17–19]. Gilts, then, represent an important population in porcine production, and so the successful application of pC-AI in gilts might involve substantial economic savings.

The main objective of this study, therefore, was to compare the implementation of pC-AI in gilts using a commonly used multiparous catheter in combination with different treatments or new catheters designed exclusively for gilts.

2. Material and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guiding Principles for the Care and Use of Animals (DHEW Publication, NIH, 80-23). The protocol was approved by the Ethical Committee for Experimentation with Animals of the University of Murcia, Spain (project number AGL2015-66341-R).

2.2. Animals

This study was carried out under field conditions in five commercial swine farms, located in south-eastern Spain (province of Murcia). *Landrace x Large White* gilts with 2 to 3 previous detected oestrus periods were used in the study. The animals were fed a commercial diet twice a day (3200 kcal/kg). Regarding the amount of feed per day a gestation curve was used. From mating to 30 days post-insemination the sows were fed 3.5 kg/day, from day 31 to 90 the amount was reduced to 2 kg. Finally, feed was increased to 3 kg from day 91 until farrowing. The barns were equipped with mechanical ventilation and evaporative cooling systems in order to control the temperature (20 ± 2 °C). The light was also controlled (14 h light/10 h darkness). Boars were individually located in pens and were fed once a day with a vitamin complement (Fertivall Verracos, Mevet, Spain) added to the fodder. Water was provided *ad libitum*. Boar replacement was 50% per year.

2.3. Experimental design

The gilts in oestrus on the day of the experiments were distributed randomly and equally among the different experimental groups in order to avoid herd and season influences in the results.

2.3.1. Experiment 1: application of pC-AI in gilts using multi-ring multiparous catheter and pharmacological (Vetrabutin Chlorhydrate-VC) or warm extender (WE) treatment

A first attempt to inseminate the females (n = 104, average weight 173.12 ± 17.27 kg) was performed using the multiparous pC catheter (MpC) [multi-ring tip (22 mm in diameter) and inner cannula (3.5 mm in diameter)] (Fig. 1a). Gilts were classified as either "inseminated" (the inner cannula was introduced easily or with little difficulty and no backflow was observed inside the cervical catheter at the moment of dose application) or "insemination not possible" (the inner cannula was impossible to insert or could be inserted but backflow was observed inside the cervical catheter at the moment of dose application). When females were classified as "insemination not possible", one of two alternatives were applied before the second pC-AI attempt (females were randomly assigned): 1) VCpC group: intramuscular (i.m.) administration of Vetrabutin Chlorhydrate (VC) (2-4 mL) (Monzal®, Boehringer Ingelheim, Spain) (n = 38; 168.5 ± 14.58 kg average weight) just after first attempt of insemination was performed. VC is routinely used to relax the uterine muscle during farrowing (uterotonic drug with vasodilator effects). Its activity is specific to the uterine body and cervical musculature; acting on the myometrial cells, sealing off the membrane against the passage of potassium ions, thereby increasing membrane potential by lowering tonus [20–22]; 2) WEpC group: cervical deposition of warm commercial extender (WE) (44.9 ± 2.15 °C in 20.1 ± 3.32 mL) (n = 42; 164.5 ± 29.99 kg average weight) just after first attempt of insemination was performed, in order to modify the muscle dynamics through the effect of temperature [23] and help cannula penetration during the second attempt. Gilts were considered successfully inseminated following the same criteria as mentioned above. Presence of blood in the catheter/cannula was also analyzed after AI was performed. Data concerning reproductive parameters [farrowing rate (%), total and live piglets born, and fecundity index (indicates total number of piglet born per 100 inseminations and calculated by: farrowing rate *x* piglets born per litter)] were collected for the experimental groups [MpC-AI group: gilts successfully inseminated at first attempt; VCpC-AI group: gilts successfully inseminated using VC (i.m. injection) treatment before second pC-AI attempt; WEpC-AI group: gilts successfully inseminated using WE treatment before second pC-AI attempt] when the insemination had been carried out. The data were compared with those obtained for conventionally inseminated gilts (C-AI) (Fig. 1b), which acted as control.

2.3.2. Experiment 2: using new pC-AI catheters, with a design based on the genital tract characteristics of gilts

Experiment 2a: pC-AI applications were compared using three different types of catheters: 1) MpC: multiparous pC catheter used as control group [multi-ring tip (22 mm in diameter) and inner cannula (3.5 mm in diameter)] with the same characteristics as experiment 1 (Fig. 1a) (n = 56, 160.23 ± 27.64 kg); 2) GpC1: gilt pC catheter with a cervical tip adapted to gilts using a multi-ring silicon catheter tip and narrower (16 mm in diameter) than the MpC catheter, avoiding closure of the cervix and, as a consequence, of the lumen space (Fig. 1c) (n = 54, 150.44 ± 18.21 kg). 3) GpC2: gilt pC-AI catheter with similar cervical tip to that of GpC1 and thinner cannula (and cannula tip) than in experiment 1 (2.5 mm in diameter) in order to adapt the size of the cannula to the smaller size of the gilt cervix (n = 63, 158.74 ± 15.47 kg) (Fig. 1d). In contrast to experiment 1, where the gilts were treated if application at the first attempt failed, in experiment 2 cannula penetration was evaluated at the first attempt for the three catheters used. Insemination was