Post-cervical compared with cervical insemination in gilts: Reproductive variable assessments

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ABSTRACT

The aim with this study was to compare cervical (CAI; 3×10^9 spermatozoa/90 mL) and post-cervical (PCAI; 1.5×10^9 spermatozoa/45 mL) artificial insemination (AI) techniques for frequency of incidences (unsuccessful or difficult probe passage, backflow, metritis and bleeding), values for reproductive variables and duration of the procedure in gilts. There were 644 gilts (255–270 days old, weighing 150 ± 5 kg) randomly assigned to PCAI ($n = 320$) and CAI ($n = 324$) groups. In total, there were 957 and 958 artificial inseminations performed in the CAI and PCAI groups, respectively (2–4 AIs/gilt). The frequency of unsuccessful or difficult PCAI probe passage/AI was 14.6% (140/958), therefore, there was a 85.7% probe passage success/AI rate (818/958). The semen backflow frequency/AI was less with PCAI than CAI (4.3% compared with 8.2%, $P < 0.001$). With the PCAI group, there were only a few cases of bleeding (11/958: 1.1% /AI) with no difference between the CAI and PCAI groups ($P = 0.224$). In gilts ($n = 72$) where there was not passage of the PCAI probe (72/320; 22.5%) there was use of CAI, (M, mixed group). For the CAI, PCAI and M groups, there were similar values for positive pregnancy diagnosis, farrowing rates and prolificacy ($P > 0.05$). The average duration for AI was shorter in the PCAI (2.34 ± 0.809 min) than CAI (4.77 ± 1.059 min) group, and it was longer in the M group (7.48 ± 2.454 min; $P < 0.050$). The PCAI procedure, therefore, is recommended for AI of gilts.

1. Introduction

Currently, most pig farms worldwide use cervical artificial insemination (CAI) in the reproductive management of gilts (Fitzgerald et al., 2008; García-Vázquez et al., 2019). For CAI, 2–3 semen doses per estrus are used, containing $2-4 \times 10^9$ sperm cells in a volume of 70–100 mL, stored at 17 °C for a maximum period of 3–7 days depending on the extender used.

The first field studies published using post-cervical artificial insemination (PCAI) were conducted by Watson and Behan (2002) with results being similar for PCAI and CAI. The PCAI procedure has been proposed as a new technique for depositing semen in the uterine body, therefore, there are fewer sperm numbers required without a decrease in productivity of the pork production enterprise

(Bennemann et al., 2004; Serret et al., 2005; Roca et al., 2011). With development of the PCAI procedure, there have been new models of catheters and probes developed (Martinez et al., 2005; Kirkwood and Kauffold, 2015; Ausejo et al., 2017; Ulguim et al., 2018; Llamas-López et al., 2019).

These AI techniques differ not only in the semen deposition site but also in the sperm concentration and dose volume used for AI. The number of doses per ejaculate for CAI is limited to 20–25 (Fitzgerald et al., 2008; Wilson, 2012; Hernández-Caravaca, 2015). A minimum volume of 50 ml containing 1.5×10^9 sperm was initially considered necessary to obtain a 91.9% farrowing rate and satisfactory litter size (Behan and Watson, 2004); therefore, each individual ejaculate can be used to produce as many as 60 doses for PCAI (Hernández-Caravaca, 2015). Boars in artificial insemination (AI) centers with the greatest genetic merit for selected traits could be used in inseminating a larger number of gilts with use of PCAI (Bortolozzo et al., 2008; Sbardella et al., 2014; Knox, 2016). The main objectives for PCAI are efficient genetic progress, minimizing semen backflow during the insemination process and decreasing the time to conduct the AI procedure, without reduction of litter size and farrowing rate (Bennemann et al., 2004; Nogueira et al., 2006; Wilson, 2012; Falceto, 2018; García-Vázquez et al., 2019).

Gilt productivity is important in pork production enterprises because gilts represent 18% of the farrowing group and produce approximately 13% of the total piglets born (Ternus et al., 2017). The use of PCAI in multiparous sows is well established, but there are only a few studies where the use of this procedure has occurred in both primiparous sows and gilts. Hernández-Caravaca et al. (2012) reported that PCAI catheters used for multiparous sows could be effectively used in only 25% of the gilts. Alternatives, therefore, are needed to implement or modify PCAI in gilts. The most important limiting factor for PCAI in gilts is the smaller reproductive tract and the need for proper training of the AI technician in the use of flexible catheters (Levis et al., 2001; Ausejo et al., 2017; Hernández-Caravaca et al., 2017; Ulguim et al., 2018). The primary objective of the present study, therefore, was to compare the effects of CAI and PCAI on fertility, farrowing rate and prolificacy variables in gilts.

2. Materials and methods

2.1. Ethical declaration

This study complied with the ARRIVE guidelines (Kilkeny et al., 2010), the Council Directive, 2008/120/EC outlining minimum standards for the protection of pigs and Directive, 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures in the experiment were conducted in ways that are consistent with the precepts of animal welfare and were approved by the Committee of Ethics in Animal Experimentation of Universidad de Zaragoza (protocol No. PI31/18).

2.2. Animals

This study was conducted using the Spanish standard commercial conditions between 2016 and 2018 on three breeding sow farms (Farms 1–3) located near Caspe (Zaragoza, Northeastern Spain, Farm 1), Fonz (Huesca, Northeastern Spain, Farm 2) and Plasencia de Jalón (Zaragoza, Northeastern Spain, Farm 3). There is a description of the characteristics of these farms in Table 1. A total of 644 gilts were used in the study; gilts were 255–270 days old, weighed 150 ± 5 kg (*SD*) and had two previously detected periods of estrus. All animals were of hyper-prolific genetic lines (Farm 1: Youna, AXIOM, Azay sur Indre, France; Farm 2: Naïma, Choice Genetics France, Bruz, France; Farm 3: DanBred, DANBRED P/S, Herlev, Denmark) and were randomly assigned to the treatment group (PCAI; $n = 320$) or a control group (CAI; $n = 324$).

The number of replicates (batches of gilts submitted to AI per week) differed among farms; the number of gilts/replicate also varied within and between farms (Table 1). Gilts were fed a commercial diet twice a day. Gilts were treated with altrenogest (REGUMATE ®, Merck & Co., Inc., Kenilworth, NJ, USA) administered orally and individually for 18 days. Water was available *ad libitum*. After AI, the gilts were housed in individual pens (0.65 × 2 m) until pregnancy status diagnosis occurred (28 days after AI). After there was a positive pregnancy diagnosis, the gestating gilts were group-housed based on week when there was AI of the gilt (about 10 gilts per pen).

2.3. Sperm collection

Semen utilized in this study was obtained from 35 different Pietrain boars (UPB®, Semen Cardona; CIA San Pedro, Cuarte S.A.; CIAR, Spain) once a week using the gloved-hand technique for collections and then filtered to remove the gel. The average number of spermatozoa was assessed by using a BRAND® counting chamber BLAUBRAND® Bürker pattern (Merck & Co., Inc., Kenilworth, NJ, USA); and spermatozoa motility, agglutination and abnormalities were analyzed using the ISAS Psus® software (PROISER R + D, Paterna, Spain). Heterospermic doses from Pietrain boars were used (two boars/dose). Only ejaculates with greater than the minimum requirements (motility > 80% and total abnormalities < 25%) were used. Immediately after evaluation, each ejaculate was fully diluted in a commercial extender at 37 °C (VITASEM®, Magapor, Ejea de los Caballeros, Spain) and stored in bag doses that contained 1.5×10^9 spermatozoa per 45 ml for PCAI or 3×10^9 spermatozoa per 90 ml for CAI. Doses were stored at 15 to 18 °C for 72 h.

Table 1

Characteristics of the farms where the study was conducted; Data are reported as percentages (counts/n) except for gilts/replicate which is reported as mean ± SD; M: unsuccessful PCAI immediately followed by CAI.

Variable		Farm		
		1 (n = 123 gilts)	2 (n = 109 gilts)	3 (n = 412 gilts)
Year				
	2016	–	100 (109/109)	–
	2018	100 (123/123)	–	100 (412/412)
Number of artificial inseminations/gilt				
	2	22 (27/123)	22.9 (25/109)	–
	3	72.4 (89/123)	77.1 (84/109)	100 (412/412)
	4	5.6 (7/123)	–	–
Season of AI				
	Spring	80.5 (99/123)	100 (109/109)	29.6 (122/412)
	Summer	19.5 (24/123)	–	70.4 (290/412)
Replicates		17	4	16
Gilts/replicate		7.24 ± 3.833	22.75 ± 18.191	23.50 ± 17.143
First to second AI interval				
	12 h	30.9 (38/123)	–	90.5(373/412)
	24 h	69.1 (85/123)	–	9.5 (39/412)
Second to third AI interval				
	12 h	66.3 (59/89)	–	8.5 (5/412)
	24 h	33.7(30/89)	–	91.5 (377/412)
Third to Fourth AI interval				
	12h	–	–	–
	24h	100 (7/7)	–	–
Group				
	CAI	54.5 (67/123)	47.7 (52/109)	49.8 (205/412)
	PCAI	33.3 (41/123)	43.1 (47/109)	38.8(160/412)
	M	12.2 (15/123)	9.2 (10/109)	11.4 (47/412)

2.4. Insemination assays

Estrous detection in gilts was performed twice daily using mature boars or on the basis of standing reflex in response to human-imposed back pressure. The occurrence of estrus was defined as the standing reflex when the back pressure method was performed, as well as reddening and swelling of the vulva. There was insemination of the gilts for the first time when estrus symptoms were detected and again at intervals of 12 to 24 h throughout the duration of the period of estrus. Data in Table 1 are the number of artificial inseminations per gilt and the AI timing distributions for two of the farms; unfortunately, no data about AI timing at Farm 2 were available. The mean number of inseminations per gilt was 2.93 ± 0.29 (SD) (minimum: 2 and maximum: 4). There was only seven gilts from Farm 1 for which there was AI four times. The gilts in the control group (CAI) were inseminated in the presence of a boar, and the semen was deposited in the cranial portion of the cervix using the foam tip catheter for gilts manufactured by Magapor (Ejea de los Caballeros, Spain). For gilts in the PCAI group, insemination was conducted without the presence of a boar, using a specific PCAI probe for gilts (MAGAPLUS N®, Magapor, Ejea de los Caballeros, Spain) and, as a guide, the foam tip catheter for gilts that was manufactured by Magapor (Ejea de los Caballeros, Spain). The only methodological difference between the PCAI and CAI techniques was the use of the specific PCAI probe for gilts with doses that contained 1.5×10^9 spermatozoa per 45 mL. The duration of time required to conduct the AI procedures was recorded for 123 and 412 gilts from Farms 1 and 3, respectively; the average duration of conducting the AI was determined for each gilt. There was recording of the frequencies of unsuccessful probe passage, difficult probe passage, backflow, bleeding and metritis at insemination. No evaluation of semen backflow or bleeding volume was conducted; therefore, both semen backflow and bleeding were considered as a qualitative variable with two values being recorded (absence/presence). There were assessments for both backflow and bleeding at the time of insemination. Both CAI and PCAI procedures in every gilt were conducted by one technician per farm. For the gilts where there could not be passage of the PCAI probe, there was immediate use of CAI, and the gilts where this occurred were allocated to a third group, termed the M group (mixed group).

2.5. Return of estrus and diagnosis of pregnancy

Pregnancy was diagnosed using trans-abdominal ultrasonography (Future-1®, Inserbo, Spain) 28 days post-insemination. The return to estrus was assessed by boar stimulation or by applying back pressure to the gilts. Returns to estrus, abortions and deaths after confirmation of pregnancy were recorded by the AI technician. Values for reproductive variables (pregnancy and farrowing rates; total number of piglets born, number of live-born piglets and number of stillborn piglets and mummies per litter) were recorded for every farrowing.

2.6. Statistical analysis

All statistical analyses were performed using SPPSS v. 22. Values for qualitative variables were analyzed by cross tabulation, and percentages were compared using the Pearson's χ^2 test and, alternatively, the Fisher´s exact test (2 × 2 tables with small effective size) was used. Binomial logistic regression was applied to binary variables for pregnancy and farrowing rate: a binomial logistic regression was performed to ascertain the effects of farm, group and number of artificial inseminations on the likelihood that there would be a positive pregnancy diagnosis and whether the gilts farrowed. The General Linear Model (GML) was used for the analyses of total number of piglets born and live-born piglets/litter. The model included farm and group as fixed effects and number of artificial inseminations as a covariable; also, the total number of born (piglets/litter) was considered a covariable for the evaluation of live-born piglets/litter. Both fixed effects and covariates were maintained in the model regardless of the effect (P value). In Table 5, there are the results from use of the complete models. Data for stillborn piglets/litter and mummies/litter were analyzed using a non-parametric tests (Kruskall- Wallis test). The average amount of time for conducting AI procedures was calculated for every gilt as the sum of the duration of insemination per number of inseminations and the Breslow's test was used for comparisons between groups and farms. Differences were considered significant at $P < 0.05$.

3. Results

Data included in Table 2 are the results when the different AI procedures (CAI and PCAI) were used. There were 72 gilts for which passage of the PCAI probe could not be accomplished (72/320; 22.5%) and that were immediately submitted to CAI. These data for the gilts of the M group are shown in Table 1 and the frequency of assigning gilts to the M group did not differ among farms ($P = 0.629$). The data for distribution of gilts of the M group on the basis of the successive AI procedures are shown in Table 3.

In Table 2, there are data for the results of the total number of artificial inseminations in the CAI (957 artificial inseminations for 324 gilts) and PCAI (958 artificial inseminations for 320 gilts) groups. The PCAI was performed without problems occurring in 79.9% of the artificial inseminations (765/958). Unsuccessful or difficult PCAI probe passage only occurred in 10.6% (102/958) and 4% (38/958) of artificial inseminations, respectively. Backflow of semen occurred less frequently in PCAI than in the CAI group (4.3% compared with 8.2%; $P < 0.001$). At the time of the second AI, there was diagnosis of metritis by the presence of purulent discharge in two gilts; semen backflow and metritis were detected in only one gilt (Table 2). Frequencies of semen backflow and bleeding, backflow and metritis, and metritis and bleeding were few in both the CAI and PCAI groups, and there were no significant differences between these two groups when values for this variable were compared ($P > 0.05$). In both the CAI and PCAI groups, there were no differences among the first, second and third artificial inseminations for problems related to semen backflow, semen backflow and bleeding, semen backflow and metritis, and metritis and bleeding frequencies ($P > 0.05$). Data for the fourth AI were not included in these comparisons due to the small number of gilts for which there was a fourth AI. In the PCAI group, there were no differences among the first, second and third artificial inseminations for unsuccessful probe passage rates ($P > 0.05$). There were differences for difficult probe passage frequency in the PCAI group ($P = 0.041$) with this frequency being less for the second than third AI, but difficult probe passage frequency for the first AI did not differ when compared with that when there was a second or third AI conducted.

Data in Table 4 are for pregnancy and farrowing rates per gilt inseminated in the CAI, PCAI and M groups. For the positive pregnancy rate status, after an adjustment for the significant effect of the number of artificial inseminations, there were no differences among farms or groups. There were also no group effects on the farrowing rate.

The data for prolificacy based on total piglets born/litter and live-born piglets/litter are included in Table 5. There was no effect of the number of artificial inseminations ($P > 0.05$) on prolificacy. For the total number of piglets born/litter, there were no differences among groups, but there were differences among farms. There was an effect of total piglets born/litter on live-born piglets/litter (P < 0.001). For live-born piglets/litter, there were no differences among farms or groups after an adjustment for the effect of the total number of piglets born/litter ($P < 0.001$). There was no differences among groups for stillborn piglets/litter (CAI: 1.67 ± 1.764, 8.6%; PCAI: 1.59 ± 1.825, 8.3%; M: 1.37 ± 1.631, 6.8%; $P = 0.677$). For mummies/litter, there were no differences among groups (CAI: 0.41 ± 0.795, 2.1%; PCAI: 0.39 ± 0.765, 2.0%; M: 0.39 ± 0.717, 2.0%; $P = 0.937$).

There were only limited data for the average amount of time required to conduct an AI, mainly from Farm 3. At this farm, the average duration of AI was 4.77 ± 1.059 min (205 gilts), 2.34 ± 0.809 min (160 gilts) and 7.48 ± 2.454 min (47 gilts) for CAI, PCAI and M groups, respectively. The average duration of AI was less in the PCAI than CAI ($P < 0.050$) group. In the M group, the average duration of AI was longer than in the CAI group ($P < 0.050$).

Table 2
Results with use of the successive AI procedures for all gilts in the CAI and PCAI groups and the total artificial inseminations per group; Data are reported as percentages (counts/*n*); Unsuccessful probe passage: there could not be passage of the probe through the cervix; Difficult probe passage: the probe was difficult to pass through the cervix.

AI procedure		Group	
		CAI	PCAI
First	*n* (gilts)	324	320
	No problems	89.8(291/324)	79.1(253/320)
	Unsucessful probe passage	0(0/324)	10.6(34/320)
	Difficult probe passage	0(0/324)	4.4 (14/320)
	Semen backflow	9.0(29/324)	5.0(16/320)
	Semen backflow & bleeding	0.3(1/324)	0(0/320)
	Bleeding	0.9(3/324)	0.9(3/320)
Second	*n* (gilts)	324	320
	No problems	90.5(293/324)	80.6(258/320)
	Unsucessful probe passage	0(0/324)	12.8(41/320)
	Difficult probe passage	0(0/324)	1.9 (6/320)
	Semen backflow	8.0(26/234)	2.5(8/320)
	Semen backflow & metritis	0.3(1/324)	0(0/320)
	Metritis	0.3(1/324)	0.3 (1/320)
	Bleeding	0.9 (3/324)	1.9 (6/320)
Third	*n* (gilts)	307	313
	No problems	92.5(284/307)	80.2(257/313)
	Unsucessful probe passage	0(0/307)	8.3 (26/313)
	Difficult probe passage	0.3(1/307)	5.8(18/313)
	Semen backflow	7.2(22/307)	5.1(16/313)
	Bleeding	0(0/307)	0.6(2/313)
Fourth	*n* (gilts)	2	5
	No problems	50(1/2)	60(3/5)
	Unsuccessful probe passage	0(0/2)	20 (1/5)
	Semen backflow	50 (1/2)	20 (1/5)
Total	*n* (AIs)	957	958
	No problems	90.8(869/957)	79.9(765/958)
	Unsuccessful probe passage	0(0/957)	10.6(102/958)
	Difficult probe passage	0.1(1/957)	4.0(38/958)
	Semen backflow	8.2(78/957)	4.3(41/958)
	Semen backflow & bleeding	0.1(1/957)	0(0/958)
	Semen backflow & metritis	0.1(1/957)	0(0/958)
	Metritis	0.1(1/957)	0.1 (1/958)
	Bleeding	0.6(6/957)	1.1(11/958)

Table 3
Distribution of gilts with use of PCAI or unsuccessful PCAI immediately followed by CAI (M) in successive AI procedures; Data are reported as percentage (count/*n*). NA: third/fourth AI was not conducted.

First AI	Second AI	Third AI	Fourth AI
PCAI: 89.3 (286/320)	PCAI: 81.2 (260/320)	NA: 1.2 (4/320)	NA: 1.2 (4/320)
		PCAI: 76.3 (244/320)	NA: 75.4 (241/320)
			PCAI: 0.9 (3/320)
		M: 3.7 (12/320)	NA: 3.7 (12/320)
	M: 8.1 (26/320)	NA: 0.6 (2/320)	NA:0.6 (2/320)
		PCAI: 5.3 (17/320)	NA: 5.3 (17/320)
		M: 2.2 (7/320)	NA: 1.9 (6/320)
			M: 0.3 (1/320)
M: 10.7 (34/320)	PCAI: 6.0(19/320)	PCAI: 5.7 (18/320)	NA: 5.3(17/320)
			PCAI: 0.3 (1/320)
		M: 0.3 (1/320)	NA: 0.3 (1/320)
	M: 4.7 (15/320)	NA: 0.3 (1/320)	NA: 0.3 (1/320)
		PCAI: 2.5 (8/320)	NA: 2.5 (8/320)
		M: 1.9 (6/320)	NA: 1.9 (6/320)

4. Discussion

As reported by Bortolozzo et al. (2015), there has been a wide range in volume (10–85 mL) and number of sperm (0.1–4 × 10⁹) used for PCAI. In the present study, PCAI and CAI techniques were different than those in previous studies not only in semen deposition site but also in sperm concentration and dose volume. In the present study, therefore, there were comparisons of the

Table 4

Pregnancy and farrowing rate per gilt inseminated in CAI, PCAI and M groups; Data are reported as percentages (counts/n); M: unsuccessful PCAI immediately followed by CAI; Pregnancy rate: proportion of inseminated females that were pregnant; Farrowing rate: proportion of inseminated females that farrowed.

Variable	Group			Effect		
	CAI ($n = 324$ gilts)	PCAI ($n = 248$ gilts)	M ($n = 72$ gilts)	Farm P	Group P	Number of AI P
Pregnancy rate	91.4 (296/324)	92.3 (229/248)	94.4 (68/72)	0.371	0.673	0.048
Farrowing rate	85.8 (278/324)	88.7 (220/248)	93.1 (67/72)	0.138	0.213	0.157

Table 5

Total number of piglets born and live-born piglets of gilts with use of different techniques for artificial insemination; Data are reported as mean ± SD; M: unsuccessful PCAI immediately followed by CAI.

Variable	Group			Effects			
	CAI ($n = 278$ litters)	PCAI ($n = 220$ litters)	M ($n = 67$ litters)	Group P	Farm P	Number of AI P	Total born P
Total piglets born/litter	18.28 ± 4.430	18.46 ± 4.380	17.79 ± 4.413	0.295	< 0.001	0.122	
Live-born piglets/litter	16.20 ± 3.938	16.51 ± 4.158	16.03 ± 3.618	0.487	0.490	0.824	< 0.001

effectiveness of both the specific PCAI probe used for gilts and small sperm concentration and dose volume.

The farms differed for important factors in this study (year, number of AIs/gilt, season, replicates, gilts/replicate, AI timing), although there were no significant differences for CAI, PCAI and M percentages. These factors were associated with farms and, therefore, could not be included in the GLM as independent factors, but the effects were grouped into the factor "farm".

The use of PCAI represents an important aspect of the developments in reproductive biotechnology that have occurred on swine farms in recent decades. The use PCAI has increased worldwide, although application is limited mainly to multiparous sows. Since 1959, when Hancock (1959) described and used nonsurgical intrauterine insemination for the first time in multiparous sows, there has been use of several types of instruments, such as endoscopes, catheters or probes and pipettes for this purpose. There are certain limitations, including the lack of suitability for application in Landrace × Large White gilts (Hernández-Caravaca et al., 2017; Llamas-López et al., 2019).

In the present study, the small frequencies of unsuccessful or difficult PCAI probe passage per AI ([102 + 38]/958 = 14.6%) resulted in a percentage of probe passage success of 85.4% per AI (818/958), a relatively greater percentage when compared to the percentages that are generally reported. Usually, in Landrace × Large White lines the percentages of success in probe passage are 95% in multiparous and 85% in primiparous (Sbardella et al., 2014; Bortolozzo et al., 2015; Hernández-Caravaca et al., 2017). The results in the present study are inconsistent with the recommendation not to use PCAI for gilts (Levis et al., 2001; Dallanora et al., 2004 Cambourough 22 line from Agroceres PIC, Rio Claro, São Paulo, Brazil), but are consistent with results of Sonderman (2016) and Ternus et al. (2017) where it was concluded that PCAI can be used in Landrace × Large White gilts without compromising enterprise pork production efficiency. In recent years, there was development of new catheters and probes to prevent injuries to the gilts while conducting AI procedures and to simplify the use of the PCAI procedure on commercial farms (García-Vázquez et al., 2019).

Most of these studies were conducted with Landrace × Large White gilts, but no detailed information about age and weight was available. Lines used in the present study had a Large White and/or Landrace ancestry and it is important to recognize that the specific cross schemes used to produce commercial gilts could lead to different animal sizes. The standard age for first insemination is usually 220–230 days of age; thus, there are apparently only minor differences in weight, therefore, weight differences could not account for the differences in results in the present compared with those in some previous studies. There was use of a specific PCAI probe for gilts (MAGAPLUS N®, Magapor, Ejea de los Caballeros, Spain) in the present study and, as a guide, the foam tip catheter was used for PDAI in the present study (Magapor; Ejea de los Caballeros, Spain). It is believed that the use of these devices in the present study that were not used in some of the previous studies could explain for the result differences between the present and previous studies.

In the PCAI group, the frequency of semen backflow per AI was less than in the CAI group. Ausejo et al. (2017); Dominiek et al. (2011) and Hernández-Caravaca et al. (2017) reported similar results in both multiparous sows and gilts (Landrace × Large White lines). Semen backflow occurs due to many factors, but it is usually explained by the AI procedure being performed incorrectly thus causing difficulty of passage and torsion of the internal catheter that results in the backflow of the semen (Ausejo et al., 2017; Ternus et al., 2017; García-Vázquez et al., 2019).

Semen backflow, semen backflow and bleeding, semen backflow and metritis, and metritis and bleeding rates did not seem to be affected by the number of artificial inseminations performed on individual gilts in the present study. For the PCAI group, only a very small number of bleeding cases were observed (11/958: 1.1% per AI) and there were no differences between the CAI and PCAI groups ($P = 0.224$). Bleeding would be related to the animal, rather than to the number of artificial inseminations. Serret et al. (2005);

Rozeboom et al. (2014) and Llamas-López et al. (2019) advised against the use of PCAI in Landrace × Large White gilts because of the small size of the reproductive tract in gilts, the use of the PCAI procedure was considered to cause cervical injuries. Results from the present study, however, indicate the use of a specific probe by a qualified technician does not result in severe lesions in the cervix of gilts, confirming the previously reported results of Ausejo et al. (2017).

Although there were differences between farms, the average duration taken to conduct the AI procedure per gilt was longer in the CAI than PCAI group. The use of a specific PCAI probe and smaller dose volume likely contributes to this difference. Similar results were reported by Ternus et al. (2017).

The differences for difficult probe passage frequency among successive artificial inseminations could not be clearly explained by the increase or decrease in this rate as the number of artificial inseminations increased. The effect of the number of artificial inseminations per gilt was significant only for the positive pregnancy diagnosis-rate status, but after an adjustment for this effect, there was no effect for farm or group.

There was an effect of farm on total number of born piglets/litter. Effects of year, number of artificial inseminations/gilt, season, replicates, gilts/replicate, AI timing and farm were grouped as a "farm" effect; therefore, it was not possible to assess the specific effect of each factor. In the present study, the most important effect on live-born piglets/litter, stillborn piglets/litter and mummies/litter was consistent with the finding for total number of born piglets/litter; after an adjustment for this significant effect ($P < 0.001$), there was no effect of farm or group.

Values for reproductive variables in the present study are consistent with those previously reported by Sonderman (2016) and Ternus et al. (2017) with there being no differences for pregnancy and farrowing rates between the CAI and PCAI groups. There were also no differences in values for prolificacy variables (total born, live-born and stillborn piglets and mummies/litter) between these two groups and there have been similar results reported by Fitzgerald et al. (2008); Hernández-Caravaca et al. (2012) and Hernández–Caravaca (2015) in multiparous sows and Sbardella et al. (2014) in primiparous gilts. Results from the present study indicate that PCAI does not compromise the reproductive performance in gilts.

5. Conclusions

In gilts, the percentage of PCI conducted without problems being incurred was 79.9% per AI (765/958) with small percentages for both unsuccessful probe passage (10.6%, 102/958) and difficult probe passage (4.0%, 38/958). The frequency of semen backflow, metritis and bleeding was also small (global frequency: 5.5%, 43/958). The use of the PCAI technique, performed with semen doses containing 1.5×10^9 sperm cells per 45 mL, resulted in reproductive and prolificacy performances similar to those observed with traditional CAI (semen doses 3×10^9 sperm cells per 90 mL). There, therefore is recommendation for use of the PCAI procedure for AI of gilts.

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Declarations of Competing Interest

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References

Ausejo, R., Mendoza, N., Dahmani, Y., Mitjana, O., Falceto, M.V., 2017. Effect of incidents associated to post-cervical artificial insemination on reproductive. Bulg. J. Vet. Med. 21, 1311–1477. https://doi.org/10.15547/bjvm.1031.

Behan, J.R., Watson, P.F., 2004. A commercially based field trial to investigate trans-cervical insemination at reduced volume and sperm concentration in sows. In: França, L.R., Godinho, H.P., Henry, M., Melo, M.I.V. (Eds.), Proceedings of the 15th International Congress on Animal Reproduction. Colégio Brasileiro de Reprodução Animal Porto Seguro. pp. 573.

Bennemann, P.E., Milbradt, E., Diehl, G.N., Weber, D., Schimidt, A.C.T., Bernardi, M.L., 2004. Reproductive performance of sows submitted to intrauterine insemination at different pre-ovulatory intervals. Anim. Reprod. 1, 106–110.

Bortolozzo, F.P., Maria, A., Goldberg, G., Wentz, I., 2008. How far is it possible to reduce the number of spermatozoa after intra- cervical insemination in swine without compromising fertility? Acta Sci. Vet. 36 (Suppl. 1), 17–26.

Bortolozzo, F.P., Menegat, M.B., Mellagi, A.P.G., Bernardi, M.L., Wentz, I., 2015. New artificial insemination technologies for swine. Reprod. Domest. Anim. 50, 80–84. https://doi.org/10.1111/rda.12544.

Council Directive 2008/120/EC of 18 December 2008, 2008. Laying down minimum standards for the protection of pigs. L 47/5-13. Off. J. Eur. Union.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, 2010. On the protection of animals used for scientific purposes. L 276/33-74. Off. J. Eur. Union.

Dallanora, D., Mezalira, A., Katzer, L.H., Bernardi, M.L., 2004. Reproductive performance of swine females inseminated by intrauterine or traditional technique. Pesqui. Agropecu. Bras. 39, 815–819. https://doi.org/10.1590/S0100-204x2004000800013.

Dominiek, M., Lopez Rodriguez, A., Rijsselaere, T., Vyt, P., Van Soom, A., 2011. Artificial insemination in pigs. In: Manafi, M. (Ed.), Artificial Insemination in Farm Animals. InTech, Rijeka, Croatia, pp. 79–94. https://doi.org/10.5772/16592.

Falceto, M.V., 2018. Essential guides on swine health and production. Artificial Insemination and Hormone Management in the Sow. Servet editorial. Grupo Asís

Biomedia, S.L., Zaragoza, pp. 46–50.

Fitzgerald, R.F., Jones, G.F., Stalder, K.J., 2008. Effects of intrauterine and cervical artificial-insemination catheters on farrowing rate and litter size. J. Swine Heal. Prod. 16, 10–15.

García-Vázquez, F.A., Mellagi, A.P.G., Ulguim, R.R., Hernández-Caravaca, I., Llamas-López, P.J., Bortolozzo, F.P., 2019. Post-cervical artificial insemination in porcine: the technique that came to stay. Theriogenology. https://doi.org/10.1016/j.theriogenology.2019.02.004.

Hancock, J.L., 1959. Pig insemination technique. Vet. Rec. 71, 523–527.

Hernández-Caravaca, I., 2015. Productive Output of Post-Cervical Insemination in Porcine. Study of Sperm Selection in the Female Genital Tract Through Backflow Analysis. Dr. Diss. Universidad de Murcia (Accessed 19 March 2019). https://www.tesisenred.net/bitstream/handle/10803/310943/TIHC.pdf?sequence=1&isAllowed=y.

Hernández-Caravaca, I., Izquierdo-Rico, M.J., Matás, C., Carvajal, J.A., Vieira, L., Abril, D., Soriano-úbeda, C., García-Vázquez, F.A., 2012. Reproductive performance and backflow study in cervical and post-cervical artificial insemination in sows. Anim. Reprod. Sci. 136, 14–22. https://doi.org/10.1016/j.anireprosci.2012.10.007.

Hernández-Caravaca, I., Llamas-López, P.J., Izquierdo-Rico, M.J., Soriano-Úbeda, C., Matás, C., Gardón, J.C., García-Vázquez, F.A., 2017. Optimization of post-cervical artificial insemination in gilts: effect of cervical relaxation procedures and catheter type. Theriogenology 90, 147–152. https://doi.org/10.1016/j.theriogenology.2016.11.027.

Kilkeny, C., Browne, W.J., Cuthill, I.C., Emewrson, M., Altman, D.G., 2010. Improving bioscience research reporting: The ARRIVE Guidelines for reporting animal research. PLoS Biol 8, e1000412. https://doi.org/10.1371/journal.pbio.1000412.

Kirkwood, R.N., Kauffold, J., 2015. Advances in breeding management and use of ovulation induction for fixed-time AI. Reprod. Domest. Anim. 50, 85–89. https://doi.org/10.1111/rda.12524.

Knox, R.V., 2016. Artificial insemination in pigs today. Theriogenology 85, 83–93. https://doi.org/10.1016/j.theriogenology.2015.07.009.

Levis, D.G., Burroughs, S., Williams, S., 2001. Use of intra-uterine insemination of pigs: pros, cons & economics. Anim. Sci. 618, 1–20. (Accessed 19 March 2019). http://digitalcommons.unl.edu/animalscifacpub/618.

Llamas-López, P.J., López-Úbeda, R., López, G., Antinoja, E., García-Vázquez, F.A., 2019. A new device for deep cervical artificial insemination in gilts reduces the number of sperm per dose without impairing final reproductive performance. J. Anim. Sci. Biotechnol. 1, 1–9. https://doi.org/10.1186/s40104-019-0313-1.

Martinez, E.A., Vazquez, J.M., Roca, J., Cuello, C., Gil, M.A., Parrilla, I., Vazquez, J.L., 2005. An update on reproductive technologies with potential short-term application in pig production. Reprod. Domest. Anim. 40, 300–309. https://doi.org/10.1111/j.1439-0531.2005.00593.x.

Nogueira, G., Wald, D., Filha, A., Kummer, R., Koller, F., Lourdes, M., Wentz, I., Pandolfo, F., 2006. Nova pipeta para inseminação intra – uterina em suínos. Cienc. Rural 36, 179–185. https://doi.org/10.1590/S0103-84782006000100027.

Roca, J., Parrilla, I., Rodriguez-Martinez, H., Gil, M.A., Cuello, C., Vazquez, J.M., Martinez, E.A., 2011. Approaches towards efficient use of boar semen in the pig industry. Reprod. Domest. Anim. 46, 79–83. https://doi.org/10.1111/j.1439-0531.2011.01828.x.

Rozeboom, K.J., Troedsson, M.H.T., Molitor, T.W., Crabo, B.G., 2014. The effect of spermatozoa and seminal plasma on leukocyte migration into the uterus of gilts. J. Anim. Sci. 77, 2201–2206. https://doi.org/10.2527/1999.7782201x.

Sbardella, P.E., Ulguim, R.R., Fontana, D.L., Ferrari, C.V., Bernardi, M.L., Wentz, I., Bortolozzo, F.P., 2014. The post-cervical insemination does not impair the reproductive performance of primiparous sows. Reprod. Domest. Anim. 49, 59–64. https://doi.org/10.1111/rda.12224.

Serret, C.G., Alvarenga, M.V.F., Cória, A.L.P., Dias, C.P., Corcini, C.D., Corrêa, M.N., Deschamps, J.C., 2005. Intrauterine artificial insemination of swine with different sperm concentrations, parities, and methods for prediction of ovulation. Anim. Reprod. 2, 250–256.

Sonderman, J.P., 2016. The advantages of post-cervical insemination (PCAI) on a sow farm. In: Proceedings of the 47th American Association of Swine Veterinarians (AASV) Annual Meeting. New Orleans. pp. 3–5.

Ternus, E.M., Vanz, A.R., Lesskiu, P.E., Preis, G.M., Serafini, L., Consoni, W., Traverso, S.D., Cristani, J., 2017. Reproductive performance of gilts submitted to post-cervical artificial insemination. Arq. Bras. Med. Vet. Zootec. 69, 777–784. https://doi.org/10.1590/1678-4162-9285.

Ulguim, R., Vier, C.M., Betiolo, F.B., Sbardella, P.E., Bernardi, M.L., Wentz, I., Mellagi, A.P., Bortolozzo, F.P., 2018. Insertion of an intrauterine catheter for post-cervical artificial insemination in gilts. Semin. Cienc. Agrar. 39, 2833–2888. https://doi.org/10.5433/1679-0359.2018v39n6p2883.

Watson, P.F., Behan, J.R., 2002. Intrauterine insemination of sows with reduced sperm numbers : results of a commercially based field trial. Theriogenology 57, 1683–1693. https://doi.org/10.1016/S0093-691X(02)00648-9.

Wilson, M.E., 2012. Differences in mating between a boar, traditional artificial insemination, and post cervical insemination. In: Proceedings of the London Swine Conference. London, Ontario. pp. 45–53.