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Reproductive performance and backflow study in cervical and post-cervical artificial insemination in sows

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ABSTRACT

The present study was developed to evaluate multiparous sow reproductive performance and backflow in post-cervical artificial insemination (post-CAI) using a reduced number of sperm than in cervical artificial insemination (CAI). The experimental groups were divided into sows inseminated by: 1) cervical artificial insemination (CAI): 3×10^9 spermatozoa/80 ml; 2) post-CAI: 1.5×10^9 spermatozoa/40 ml (post-CAI 1); 3) post-CAI using 1×10^9 spermatozoa/26 ml (post-CAI 2). Post-CAI 1 reproductive parameters were similar to those of post-CAI 2 (except for live born litter size which was greater in post-CAI 1) and better than for the CAI group ($p < 0.01$). In a second experiment the backflow volume, number of sperm, and sperm quality in the backflow were studied in the 3 experimental groups. The % of volume and spermatozoa in the backflow was higher in the CAI group ($p < 0.05$) than post-CAI groups (statistically similar between them). Moreover, the quality parameters (motility, progressive motility, viability, chromatin decondensation and morphology) in backflow semen were identical in all three experimental groups, but differed as regards the original insemination dose incubated inside a colostomy bag (sperm quality control group). The present study shows that the use of post-CAI (either post-CAI 1 or 2) in field conditions can be recommended because the efficiency is similar (in the case of post-CAI 2) or higher (post-CAI 1) than when using the traditional method (CAI), representing a reduction cost.

1. Introduction

Commonly, porcine insemination involves depositing the semen dose within the posterior portion of the cervical canal (approximately 15 cm deep into the cervix) by means of a catheter that engages the folds of the cervix. In currently used procedures, billions of spermatozoa are used ($2.5–4 \times 10^9$ per insemination) in a large extender volume (70–100 ml). Sperm concentration and dose volume affect the efficiency in an artificial insemination (AI) system. A reduction in the number of sperm per dose would result in a higher number of doses produced per boar with considerable economic savings (Levis et al., 2002) since it would mean more available doses to service a higher number of females (Rozeboom et al., 2004).

New strategies have been developed to improve the results obtained in AI using a lower number of spermatozoa per dose. The basic idea of these techniques is to deposit the semen closer to the site of fertilization using a lower number of sperm and volume than usual. These methods avoid

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the transit of spermatozoa through most of the female tract and there is no evidence that sperm transport in intrauterine insemination is more efficient in the presence of a great volume of fluid (Mezalira et al., 2005). Two methods of intrauterine AI are currently available: post-cervical insemination (post-CAI) (Gil et al., 2000, 2004; Watson and Behan, 2002; Roberts and Bilkei, 2005) and deep intrauterine insemination (DUI) (Krueger and Rath, 2000; Martínez et al., 2001). The difference between both techniques is mainly the place where the semen is deposited: in post-CAI the sperm is deposited in the uterine body, whereas in DUI the sperm is placed in the proximal segment of one uterine horn. Both systems permit a reduction in the number of spermatozoa and volume of inseminated dose compared with CAI. DUI allows the seminal doses to be reduced to 0.15×10^9 spermatozoa per insemination in 5 ml extender (Dimitrow et al., 2007). Recently, a novel double uterine semen deposition insemination method was seen to provide encouraging results (Mozo-Martín et al., 2012). However, so far, only post-CAI has been adapted for application in field conditions (Roberts and Bilkei, 2005).

Prior to their encounter with the oocytes and their participation in fertilization, spermatozoa are sequentially exposed to various environments along the female genital tract, where they pass rapidly through the cervix and uterus but spend lengthy times within the oviduct (Rodríguez-Martínez, 2007). When AI is used in the pig, spermatozoa are deposited in the narrow cervical canal, so that they rapidly enter the uterine cavity (Rodríguez-Martínez, 2007). A small subpopulation of spermatozoa is rapidly (within min) (Hunter, 1981) transported by myometrial contractions toward the utero-tubal junction colonizing the tubal sperm reservoir (reviewed by Rodríguez-Martínez et al., 2001, 2005), before the most of the remaining spermatozoa are cleansed from the genital tract either through intrauterine phagocytosis by incoming polymorphonuclear neutrophils or by way of retrograde flow (backflow) (Steverink et al., 1998; Matthijs et al., 2000, 2003). The backflow rate depends mainly on the site of semen deposition and the volume of the seminal dose (Baker and Degen, 1972). These have been several backflow studies in porcine (Steverink et al., 1998; Matthijs et al., 2003; Mezalira et al., 2005), but none has compared backflow in CAI and post-CAI in the same conditions.

Several studies have shown that spermatozoa are able to interact with their environment and that such interaction has an impact on sperm viability (reviewed by Taylor et al., 2008). Although the uterus function (cervix and uterus body) as regards sperm after insemination or mating has not yet been thoroughly investigated, this organ could play an important role by acting as the first sperm quality control step before they go more deeply into the reproductive track, discarding the less competitive sperm in the backflow. A study of the quality of semen backflow could help clarify this.

The objectives of our study were: 1) to compare reproductive parameters (return to oestrus %, abortion %, pregnancy %, farrowing % and litter size) in CAI and post-CAI groups using different sperm concentrations and volumes in the insemination dosage (CAI: 3×10^9/80 ml; post-CAI 1: 1.5×10^9/40 ml and post-CAI 2: 1×10^9/26 ml); 2) to evaluate the backflow volume (%) and number of spermatozoa (%) in the backflow in the 3 insemination groups described above and analyze backflow sperm quality (motility %, progressive motility, viability %, chromatin decondensation and morphology %) compare with the original sperm dose kept in the same conditions in which the backflow was collected in order to analyze whether the uterus provokes a sperm selection.

2. Material and methods

2.1. Animals and sperm collection

This study was carried out under field conditions at the commercial pig farm "Agropecuaria El Escobar", Murcia (South-Eastern Spain). Multiparous sows (Landrace × Large White) from parity 2 to ≥7 and 20 boars (Duroc) of proven fertility were used in this study. The sows were maintained in individual crates and were fed a commercial diet twice a day. The swine houses were equipped with mechanical ventilation and evaporative cooling systems in order to control the temperature ($20 \pm 2\,^{\circ}$C). The photoperiod was also controlled (14 h light/10 h darkness). Boars were individually located in stables and were fed once a day with a vitamin complement added to the fodder. Water was provided *ad libitum*.

Each boar was used to obtain semen once a week using the gloved-hand technique and filtered to remove the gel. The average number of spermatozoa/AI dose was controlled in a haemocytometer (Neubauer counting chamber; VWR International, Haasrode, Belgium). Only ejaculates with a rich fraction volume ≥75 ml, concentration $\geq 200 \times 10^6$ sperm/ml, motility ≥70% and total abnormalities ≤20% were used in this study. Immediately after evaluation, each ejaculate was fully diluted with *Beltsville Thawing Solution* (BTS, Minitüb, Tiefenbach, Germany) and stored in 80, 40, 26 ml bottles containing 3×10^9, 1.5×10^9 and 1×10^9 spermatozoa, respectively. Doses were stored at 16° C and used within 24 h. Heterospermic doses (from two boars with an equal sperm number per boar) were prepared and proportionally divided to inseminate the sows. The ejaculates of all the boars were used approximately in the same proportion with all sows and insemination methods.

2.2. Insemination assays

Multiparous sows used for breeding were weaned 28 days after farrowing. Oestrus detection was performed twice daily by experienced workers by allowing sows nose-to-nose contact with mature boars and applying back pressure. The occurrence of oestrus was defined by the standing reflex in front of a teaser boar and reddening and swelling of the vulva.

Cervical AI (CAI) was performed with disposable spiral tip catheters (Inserbo S.L., Lérida, Spain). Sows were inseminated 12 and 24 h after oestrus detection (2 inseminations per sow), using 3×10^9 spermatozoa per dose in 80 ml of BTS. After thorough cleaning and drying the sow's vulvar labia area, a spirette was inserted through the vagina into the cervix to produce a cervical lock. Back pressure was

applied and the seminal dose was allowed to flow into the uterus applying a constant pressure in the dose tube. The insemination dose was introduced gently and slowly in the sow's uterus to reduce the backflow. The catheter remained in the cervix 2–4 min after insemination to reduce the backflow.

Post-cervical AI (post-CAI) was performed with a combined catheter-cannula kit (Soft & Quick®, Import-vet, SA, Barcelona, Spain), which consists of a 72 cm flexible cannula inserted into a conventional cervical catheter. Sows were inseminated 12 and 24 h after oestrus detection (2 inseminations per sow). Doses of 1.5×10^9 sperm in 40 ml (post-CAI 1 group) and 1×10^9 sperm in 26 ml (post-CAI 2 group) were used in two different groups of sows. The sperm dose must be introduced quickly (only a few seconds). The inner catheter was removed and then, with the cervical catheter still placed in the cervix and shaken in a rotational way, the neck of the womb was massaged for five seconds after which the catheter was removed.

The three inseminations were performed by four technicians (each technician applied the three AI methods in the same proportion).

The return to oestrous was evaluated using male sexual stimulation from 18 to 24 days after insemination. Abortion was detected by ultrasound monitoring (Echoscan 500, Impor-vet SA, Barcelona, Spain) in the case of early abortion or by a technician's direct visualization (late abortion). Pregnancy was detected by ultrasound 28 days after inseminations.

2.3. Backflow assays

The semen backflow was collected in human colostomy bags (fixed around the vulva and secured with tape) at the moment of insemination and for 60 min thereafter. If a sow urinated into the colostomy bag or if the colostomy bag was damaged, the corresponding value was deleted from the data.

2.3.1. Backflow volume and sperm concentration assay

The colostomy bag was emptied into a graduated tube to measure the volume, while the sperm concentrations were assessed in triplicate per sample using a Neubauer counting chamber.

2.3.2. Analysis of seminal motion parameters

Percent motility and progression were determined by placing two sample aliquots on warm glass slides (39 °C) and examined under light microscopy (100× magnification). The percentage of sperm motility was estimated and forward progressive motility using an arbitrary scale from 0 to 5.

2.3.3. Viability

Viability was assessed by incubation in a solution containing 20 μl of carboxyfluorescein diacetate (CFD), to which 20 μl of propidium iodide (PI), 10 μl of formaldehyde saline solution (1%), 100 μl of the sample of semen and 900 μl of SSF were added (Harrison and Vickers, 1990). This suspension was evaluated using a microscope equipped with epi-fluorescence (100X magnification, Leica® DMLS), on at least 200 cells per sample. We classified observed spermatozoa into two groups: 1) cells with green fluorescence: intact membrane (viable); and 2) cells with red fluorescence: altered membrane (non-viable).

2.3.4. Spermatozoa morphology analysis

Wet mounts of semen fixed in buffered 2% glutaraldehyde solution were examined under a phase-contrast microscope (100× magnification) to analyze morphology. Two hundred spermatozoa were categorized according to sperm morphology into those with normal morphology, cells with attached cytoplasmic droplets, tail defects (folded tail, coiled tail) and others (abnormal heads, *etc.*).

2.3.5. Determination of chromatin condensation by flow cytometry

Flow cytometric analyses were performed on a Coulter Epics XL cytometer (Beckman Coulter Inc., Miami, FL, USA). A 15 mW argon ion laser operating at 488 nm excited the fluorophores. Data from 10,000 events per sample were collected in list mode, and four measures per sample were recorded. Flow cytometric data were analyzed using the program Expo32ADC (Beckman Coulter Inc.) and a gate in forward and side scatter to exclude remaining debris and aggregates from the analysis.

Sperm chromatin was stained with PI to determine sperm chromatin condensation. Sperm samples were centrifuged (1200 $g \times 3$ min) and the pellet was re-suspended in a solution of phosphate-buffered saline (PBS):ethanol (30/70 v/v) for 30 min for sperm membrane permeabilization and stored at −20 °C until analysis. After thawing, the samples were centrifuged and the supernatant was discarded and the pellet was re-suspended in a PI solution (PI, 10 mg/ml) in PBS. Samples were maintained in darkness for 1 h before flow cytometric analysis. Red PI fluorescence was collected with a FL3 sensor using a 650 nm band-pass filter. Measurements were expressed as mean red intensity fluorescence units (mean channel in the FL3), which was used as an index of the state of the chromatin condensation, as this parameter is directly related with PI uptake by DNA.

3. Experimental design

3.1. Experiment 1. Reproductive parameters in cervical and post-cervical AI

Sperm concentration, volume and deposition place were compared in the 3 experimental groups: CAI (3×10^9 sperm cells/80 ml), post-CAI 1 (1.5×10^9 sperm cells/40 ml) and post-CAI 2 (1×10^9 sperm cells/26 ml). A total number of 5063 sows were randomly divided into CAI ($n = 1716$), post-CAI 1 ($n = 1664$) and post-CAI 2 ($n = 1683$). The studied reproductive parameters were return to oestrous %, abortion %, pregnancy %, farrowing %, total born litter size and live born litter size. In the first part of the study, reproductive parameters were compared between insemination groups. In the second part, the relation between insemination methods and parity numbers (2–3, 4–5 and ≥6 parity groups) on farrowing %, total born, and live born litter size was studied.

Table 1
Reproductive parameters obtained for sows inseminated by CAI (3×10^9 spermatozoa), post-CAI 1 (1.5×10^9 spermatozoa) and post-CAI 2 (1×10^9 spermatozoa).

	CAI	Post-CAI 1	Post-CAI 2
Number of sows (*n*)	1716	1664	1683
Return (%)	6.47 ± 0.24^a	4.57 ± 0.20^b	5.88 ± 0.23^{ab}
Abortion (%)	2.68 ± 0.16	2.52 ± 0.15	2.79 ± 0.16
Pregnancy (%)	88.58 ± 0.31^a	91.65 ± 0.27^b	90.37 ± 0.29^{ab}
Farrowing (%)	82.34 ± 0.38^a	86.84 ± 0.33^b	84.08 ± 0.36^{ab}
Total born litter size (n)	13.65 ± 3.14^a	14.13 ± 3.05^b	13.87 ± 3.34^{ab}
Live born litter size (n)	12.19 ± 3.20^a	12.59 ± 3.12^b	12.16 ± 3.32^a
Fecundity index* (*n*)	1003.72	1093.31	1022.41

a, b Different superscripts in the same row indicate significantly different values ($p < 0.01$).
* Fecundity index (not included in statistical analysis): farrowing rate multiplied by average number of live piglets born per litter *(total number of live piglets born per 100 inseminations).*

3.2. Experiment 2. Study of backflow in cervical and post-cervical AI

The study was conducted in 5 replicates, using a total of 176 sows were used. The volume and number of spermatozoa in the backflow were measured in the 3 insemination groups (CAI, post-CAI 1 and 2) described. The data provided are related to % of the inseminated dosage. In addition, the sperm quality [motility %, progressive motility (0–5 scale), viability %, chromatin decondensation, and morphology %] was analyzed in the 3 experimental groups and in the original sperm dose used to inseminate which was also incubated in the colostomy bags under the same conditions in which the backflow was collected (60 min).

3.3. Statistical analysis

All statistical analyses were performed using SPSS v. 15. Data are expressed as the mean ± SEM and were analyzed by one-way ANOVA. When ANOVA revealed a significant effect, values were compared using the least significant difference pair wise multiple comparison *post hoc* Test (Tukey). Differences were considered statistically significant at $p < 0.05$.

4. Results

4.1. Experiment 1. Reproductive parameters in cervical vs. postcervical AI

Animals inseminated by post-CAI 1 showed significantly higher fertility parameters (pregnancy %, farrowing %, total born litter size and live born litter) than those inseminated by CAI. No differences were observed between post-CAI 2 and CAI groups. The oestrus return rate was higher for sows inseminated by the CAI method ($6.47 \pm 0.24\%$) than post-CAI 1 ($4.57 \pm 0.20\%$, Table 1) ($p < 0.05$). Both post-CAI methods reached similar return rates. The average percentage of sows removed during the study due to illness, accident or death was 7.2 ± 0.25, with no statistically significant differences between groups. The comparison between both post-CAI methods (post-CAI 1 *vs.* post-CAI 2) showed that when sperm number is decreased (1.5×10^9 *vs.* 1×10^9), only the number of piglets born alive is slightly decreased (12.59 ± 3.12 and 12.16 ± 3.32 respectively, $p < 0.01$) (Table 1). In addition, if the fecundity index (farrowing rate × number of piglets born alive per litter) is calculated, the post-CAI 1 group shows a higher number of piglets born alive per 100 inseminations (1093.31) than the post-CAI group 2 (1022.41) and CAI group (1003.72) (Table 1). To explore differences in the yields of the insemination techniques, the sows were grouped by parity (2–3, 4–5 and ≥6 parity groups). The influence of insemination method and parity number on fertility parameters was studied (Table 2). When 2–3 parity sows were analyzed, the best results were found for the post-CAI methods (1 and 2) (Table 2A); the fact that there were no differences between the post-CAI groups means that using a reduced number of sperm (1×10^9) leads to a similar number of total piglets born to when a higher number (1.5×10^9) is used. The fertility parameters for forth- and fifth-parity animals were not affected by the type of insemination (Table 2B). Sows in the sixth parity or higher reached the highest farrowing rate and total size born when they were inseminated by post-CAI 1 (Table 2C).

4.2. Experiment 2. Study of backflow in cervical vs. postcervical AI: volume (%), sperm (%), and sperm quality

A total of 176 sows were used, but 62 of them (35.2%) had urinated at some stage during the collection and the bags were discarded for the calculation of backflow. In general terms, backflow occurred from the beginning of insemination in CAI while in post-CAI groups it mainly appeared after approximately 15 min.

Volume of the semen backflow: only in 9 cases (7.9%) of the 114 sows was no backflow found (4 sows in post-CAI 1 and 5 in post-CAI 2). Backflow volume varied widely in all the experimental groups (CAI: 21.87-97.50%; post-CAI 1: 0–92.50%; post-CAI 2: 0 to 81.48%) (Table 3). There were no differences in the volume (%) collected between post-CAI groups (post-CAI 1: $39.39 \pm 4.14\%$ and post-CAI 2: $37.73 \pm 3.74\%$, $p > 0.05$, Table 2), but it was statistically higher in the CAI group ($54.28 \pm 3.85\%$, $p < 0.05$, Table 3). The highest volume (%) in backflow was found in sows with 2–3 parities ($p < 0.05$).

Number of spermatozoa in the semen backflow: the sperm cell concentration (% of the inseminated dosage) was affected by sperm dosage and the insemination place ($p < 0.05$). The average concentration of the spermatozoa in the backflow compared to the inseminated dosage was 25.15 ± 3.02^a %, 15.88 ± 2.24^b % and 15.21 ± 2.43^b % in CAI,

Table 2
Reproductive parameters obtained for sows with 2–3 (A), 4–5 (B) and ≥6 (C) parities inseminated by CAI (3×10^9 spermatozoa), post-CAI 1 (1.5×10^9 spermatozoa) and post-CAI 2 (1×10^9 spermatozoa).

(A)

Groups	*N*	Farrowing (%)	Total born litter size (*n*)	Live born litter size (*n*)
CAI	605	78.18 ± 0.41[a]	13.56 ± 3.17[a]	12.23 ± 3.30[a]
Post-CAI 1	651	86.94 ± 0.33[b]	14.36 ± 3.02[b]	13.00 ± 3.09[b]
Post-CAI 2	623	82.50 ± 0.38[ab]	14.33 ± 3.28[b]	12.82 ± 3.28[b]

(B)

Groups	*N*	Farrowing (%)	Total born litter size (*n*)	Live born litter size (*n*)
CAI	537	85.29 ± 0.35	14.04 ± 3.00	12.54 ± 3.07
Post-CAI 1	445	87.87 ± 0.32	14.19 ± 3.12	12.58 ± 3.28
Post-CAI 2	479	82.88 ± 0.37	13.95 ± 3.32	12.08 ± 3.30

(C)

Groups	*N*	Farrowing (%)	Total born litter size (*n*)	Live born litter size (*n*)
CAI	574	83.97 ± 0.36	13.34 ± 3.20[a]	11.80 ± 3.17[ab]
Post-CAI 1	568	85.92 ± 0.34	13.82 ± 3.00[b]	12.09 ± 2.96[b]
Post-CAI 2	581	86.75 ± 0.33	13.33 ± 3.34[a]	11.56 ± 3.26[a]

a, b Different superscripts in the same column and for the same parity number indicate significantly different values ($p < 0.01$).

post-CAI 1 and 2, respectively (Table 3). The highest percentage of spermatozoa in the backflow was found in sows with 2–3 parities ($p < 0.05$).

Sperm quality in the sperm backflow (motility, progressive motility, viability, chromatin decondensation and morphology): the results showed a significant difference in progressive motility, viability, and normal morphology between the original dose group and the 3 insemination groups, in which these parameters were lower ($p < 0.05$, Table 4A and B). When motility (%) was compared in the four experimental groups, the highest value corresponded to the original dose group, in which it was statistically similar to the CAI group (75.00 ± 0.00 *vs.* 67.08 ± 2.59%, respectively, $p > 0.05$) (Table 4A), but different ($p < 0.05$) from the post-CAI groups (post-CAI 1: 61.97 ± 3.42 and post-CAI 2: 61.25 ± 2.65%), although no differences were found between the three backflow groups ($p > 0.05$) (Table 4A). As regards sperm chromatin decondensation, the highest value was found in the post-CAI 2 group in which it differed significantly from original dose ($p < 0.05$). No differences were found for this parameter between the three backflow groups ($p > 0.05$) (Table 4A).

5. Discussion

The main goal during mating or AI is that an adequate population of spermatozoa reach the site of fertilization during the peri-ovulatory period. The pig AI industry hopes to achieve a reduction in costs by decreasing the number of spermatozoa inseminated per dose while maintaining the same efficiency in terms of pregnancy rate and litter size as afforded by traditional insemination.

Analysis of reproductive parameters (Table 1) shows that all of them were affected except the abortion rate, which was similar in the 3 experimental groups. As regards the return rate the lowest value was obtained in the post-CAI 1 group. Watson and Behan (2002) demonstrated that deep insemination has no effect on the uterine environment with associated embryonic or fetal loss, and even improves the chance of conception and the maintenance of pregnancy with reduced sperm dose.

In our study, the insemination dose could be reduced 2 and 3-fold in post-CAI as a result of placing the sperm close to the uterus corn bifurcation (post-CAI) compared with cervical deposition (CAI). The farrowing and litter size data obtained using post-CAI methods 1 and 2 were comparable to, or even better, than the CAI results. When insemination is intrauterine, although the sperm number used is lower than in cervical deposition, the spermatozoa are close to the place of fecundation and consequently the path taken to reach the oviduct is shorter. This agrees with earlier reports which showed that post-CAI using a reduce number of sperm cells does not negatively influence the reproductive performance compared with sows receiving conventional CAI (Watson and Behan, 2002; Dallanora et al., 2003; Roberts and Bilkei, 2005; Dimitrow et al.,

Table 3
The volume and number of spermatozoa in the semen backflow (mean ± SEM and range) during insemination in the in CAI and postCAI (1 and 2) groups, expressed as a percentage of the inseminated dosage.

Groups	Backflow volume (%)	Range (min–max)	Sperm in backflow (%)	Range (min–max)
CAI (36)	54.28 ± 3.85[a]	21.87–97.50	25.15 ± 3.02[a]	2.91–77.05
Post-CAI 1 (37)	39.39 ± 4.14[b]	0–92.50	15.88 ± 2.24[b]	0–49.95
Post-CAI 2 (41)	37.73 ± 3.74[b]	0–81.48	15.21 ± 2.43[b]	0–51.68

a, b Letters in the same column indicate significant differences ($p < 0.05$).

Table 4
Sperm quality in backflow after CAI and postCAI (1 and 2). A) Motility (%), progressive motility (0–5), viability (%) and chromatin decondensation (arbitrary fluorescence units). B) Sperm morphology. Original dose: sperm incubated for 60 min in colostomy bag. Data expressed as mean ± SEM.

(A)

Groups	Motility (%)	Progressive motility	Viability (%)	Chromatin decondensation
Original dose (25)	75.00 ± 0.00[a]	3.00 ± 0.00[a]	90.44 ± 0.10[a]	17.47 ± 0.28[a]
CAI (36)	67.08 ± 2.59[ab]	2.29 ± 0.12[b]	80.56 ± 1.81[b]	39.51 ± 2.85[ab]
Post-CAI 1 (33)	61.97 ± 3.42[b]	2.16 ± 0.11[b]	83.93 ± 1.42[b]	45.36 ± 3.28[ab]
Post-CAI 2 (36)	61.25 ± 2.65[b]	2.01 ± 0.12[b]	81.23 ± 2.04[b]	69.86 ± 4.06[b]

(B)

Groups	Normal morphology (%)	Proximal cytoplasmic droplet (%)	Distal cytoplasmic droplet (%)	Altered tail (%)
Original dose (25)	81.60 ± 1.52[a]	1.68 ± 0.30[a]	13.36 ± 0.60[a]	3.04 ± 0.61
CAI (36)	66.17 ± 2.89[b]	3.09 ± 0.41[b]	26.64 ± 2.88[b]	4.26 ± 0.63
Post-CAI 1 (33)	69.30 ± 3.43[b]	2.94 ± 0.38[ab]	26.46 ± 2.72[b]	4.09 ± 0.99
Post-CAI 2 (36)	63.56 ± 3.65[b]	1.94 ± 0.31[ab]	28.86 ± 3.10[b]	5.61 ± 0.86

a, b Letters in the same column indicate significant differences ($p < 0.05$).

2007). However, when the insemination dose was reduced to 0.5×10^9 spermatozoa a decrease in pregnancy rate (to 77.3%) was found (Wolken et al., 2002).

When the reproductive parameters are analyzed according to parity (Table 2), it can be seen that post-CAI 2 is recommended in sows with 2–5 parities. When sows with 4–5 parities were used the reproductive results were similar in all the insemination groups but the use of post-CAI 2 is more economical because of the low number of sperm deposited. When ≥6 parities sows are inseminated the use of post-CAI 1 is indicated instead of the other groups since the reduction in uterine contractility prolongs the transit phase in the uterus, reducing sperm quality and thus reducing the ability of sperm cells to enter the oviducts and to fertilize (Langendijk et al., 2005). In order animal's sperm transport is reduced, perhaps because the decrease in uterine muscle tone with higher parity (Bille et al., 1974) means that more sperm is necessary in post-cervical deposition to attain the same reproductive performance.

In addition, and from an economic point of view, use of the post-CAI method would ensure important savings. Taking into account the insemination cost and total cost/sow/year (Table 5), the post-CAI technique leads to a cost reduction compared with CAI. Our analysis shows that post-CAI 2 could save 4.09 €/sow/year in comparison with post-CAI 1. However, if we take into account the significant difference in litter size the lowest piglet production cost is obtained using post-CAI 1 which result in a saving of 1.06 € and 0.77 € per weaned piglet in comparison with CAI and post-CAI 2, respectively (Table 6).

Another advantage of a lower number of spermatozoa per dose is the possibility of increasing the number of insemination doses produced per male. In current commercial conditions, one boar can produce up to 2000 doses per year with 3 billion sperm cells (Mezalira et al., 2005). By reducing the sperm number to 1000 million per dose, using the post-CAI method, the number of doses could increase by up to 300%. In addition, the number of boars per farm could also be reduced, saving on the costs associated with buying and maintaining them. This is also related with a reduction in the coefficient of variation (CV), which measures the variation from average performance (Patience et al., 2004). The application of post-CAI method increases the number of siblings in the same farm and, as a consequence, the CV falls.

Several studies have observed the same number of spermatozoa in the crypts and in the caudal isthmus region using both post-CAI (1×10^9 spermatozoa) and CAI (3×10^9 spermatozoa) methods (Sumransap et al., 2007; Tummaruk and Tienthai, 2010). Some of the principal reasons for loss of spermatozoa after insemination are backflow (Viring and Einarsson, 1981; Steverink et al., 1998; Matthijs et al., 2003) and the phagocytosis by polymofonuclear neutrophils (PMN), which influx the porcine uterus after insemination (Lovell and Getty, 1968; Pursel et al., 1978; Rozeboom et al., 1999; Matthijs et al., 2003). During natural mating approximately one-third of the spermatozoa in the ejaculate is lost through backflow within 2 h after mating (Viring and Einarsson, 1981). Clearance of the uterus after insemination has been observed in several mammalian species and is believed to be a normal physiological process, serving to prepare the uterus for the reception of the embryo or embryos (Matthijs et al., 2003).

In our study, the volume (%) and number of spermatozoa (%) in the backflow were lower in post-CAI groups than in the CAI group, which could be related with several factors. One such factor is the volume and sperm number used. As we have mentioned in this manuscript, post-CAI uses a lower volume and number of sperm than CAI, which could mean that fewer estrogens (present in the sperm and seminal plasma) are inseminated. Estrogens in the ejaculate of a boar can produce prostaglandin release by the endometrium (Claus, 1990), and prostaglandins produce an increase of myometrial activity in the uterus (reviewed by Langendijk et al., 2005). The stimulation of contractility can improve sperm transport but may also increase the time needed for the uptake of semen during insemination, as a consequence, semen backflow (Langendijk et al., 2002), as our results suggest. The reduction in backflow using post-CAI could be related with a reduction in myometrial activity because of the decreased levels of estrogens. One of the other factors involved in backflow could be the deposition site and methodology used to carry out AI. As indicated in the Material and Methods section, CAI needs to be carried out more slowly than post-CAI (2.76 ± 0.63 min *vs.* 1.12 ± 0.05 min, respectively, *data not shown*) mainly

Table 5
Evaluation of the economics of using CAI *vs.* post-CAI in field conditions.

	CAI	Post-CAI 1	Post-CAI 2
Farrowing rate (%)[a]	82.34	86.84	84.08
Inseminations/sow/year[b]	2.85	2.70	2.79
Catheter cost (€)[a]	0.15	0.60	0.60
Inseminations per cycle[a]	2	2	2
Dosage cost (€)[a]	4.00	2.80	1.96
Total insemination cost/sow/year (€)[a,c]	23.66	18.36	14.28
Fixed costs (€)[d]	681	681	681
Total cost/sow/year (€)[e]	704.66	699.36	695.28
€ saved/sow/year using post-CAI	0.00	5.29	9.38

[a] Data collected from our study.
[b] Calculated as: 2.35 farrows/sow/year × 100/farrowing rate (%). 2.35 was obtained as an average from www.sipconsultor.com-Interpig 2010 report.
[c] Total cost calculated as follows: inseminations/sow/year + catheter cost (X2) + dosage cost (X2).
[d] Fixed costs: feed, medication, replacement, workers. . .Data base collected from 25% of total sows herds in Spain 2011 (www.sipconsultor.com).
[e] Calculated as: total insemination cost/sow/year + fixed cost.

because of the lower volume used in post-CAI and the dose influx can be very fast (few seconds), because the folds of the cervix are not present, and the sperm are released close to the fertilization site. In addition when the CAI method is used the catheter must remain in the uterus an additional few minutes after insemination to minimize backflow. Zerobin and Spörri (1972) observed that contractions in the caudal part of the uterus (CAI deposition) obstructed the infusion of semen. An increased frequency of contractions probably delays the influx of semen into the caudal part of the cervix and even increases the risk of backflow (Langendijk et al., 2005).

In relation with CAI a backflow volume ranging from 0 to 120% and backflow sperm 0.3 to 79% have been reported (Steverink et al., 1998; Matthijs et al., 2003), while in post-CAI, the published data mention 14.6–22.7% sperm in the backflow (Dallanora et al., 2004; Mezalira et al., 2005). As can be observed, the backflow data found in this study (Table 3) are very similar to other previous reports, although none of these reports compared backflow in CAI and post-CAI as we have.

The backflow varied between sows of different parities, perhaps as the result of the difference in the age and size of the animals. The highest rate in volume and sperm backflow was found in the animals with 2–3 parities. Steverink et al. (1998) reported that animals with the fewer parities lost more volume than those with higher parities. Multiparous animals have a large reproductive tract which could facilitate a greater retention of fluid in the tract due to gravity (Steverink et al., 1998) and a lower capacity of uterine contractibility. However, in our backflow study approximately the same number of sows with the same age was used in the 3 experimental groups. The backflow results also pointed to a high degree of variability between sows in the same parity. One possibility to explain this finding that backflow could depend on ovulation time. During oestrus, it has been well demonstrated that sows show myometrial activity and the frequency and amplitude of contractions are maximal in this period (review by Langendijk et al., 2005). As in previous reports (Steverink et al., 1998; Mezalira et al., 2005), backflow could depend on ovulation time because this is the period when myometrial activity is greatest. However the causes of these variations in backflow between sows are still poorly understood (Steverink et al., 1998).

As mentioned above, another important reason for dosage loss is the presence of PMN in the uterus. Several factors may contribute to the recruitment of PMNs in pigs. For example, Rozeboom et al. (1998, 1999) found that extender alone could elicit an early response, while spermatozoa triggered an additional recruitment 12 h after insemination. In contrast, Engelhardt et al. (1997) reported that seminal plasma, and not spermatozoa, triggered the influx of PMNs into the stroma and epithelium of the endometrium of sows. This information suggests that as the sperm dose decreased (as in post-CAI) the presence of PMNs should diminish. In fact, Mathjins et al. (2003) observed that sows in a group receiving a low inseminate volume (20 ml) had a significantly higher number of nonphagocytosed spermatozoa in the uterus; in another

Table 6
Economic comparison between CAI and post-CAI methods in terms of cost of the weaned piglet.

	CAI	Post-CAI 1	Post-CAI 2
Total cost/sow/year (€)	704.66	699.36	695.28
Born alive[a]	12.19	12.59	12.16
Weaned/farrow[b]	10.97	11.33	10.94
Productivity/sow/year (€)[c,d]	25.78	26.62	25.71
Total cost of the weaned piglet (€)[e]	27.33	26.27	27.04

[a] Data collected from our study.
[b] Data collected from our study taking into account 10% piglet mortality during lactation.
[c] Farrows/sow/year was taken as an average (2.35) (data collected from www.sipconsultor.com-Interpig 2010 report).
[d] Weaned/farrow × Farrows/sow/year (2.35).
[e] (Total cost/sow/year)/(Productivity/sow/year).

report, a lower number of neutrophils and plasma cells in the oviduct was observed in a DUI group compared with CAI (Tummaruk and Tienthai, 2011).

The fact that only several thousand of spermatozoa reach the oviduct after the deposition of billions during insemination (Matthijs et al., 2003) suggests that, besides backflow losses and even before entering the oviduct, spermatozoa may be subjected either to a rigid selection or unspecific clearance (Taylor et al., 2008). Under normal circumstances a low number of spermatozoa is sufficient for fertilization, and these establish themselves in the oviduct during the first hour after insemination (Hunter, 1981). When the sperm quality in the backflow was analyzed, the results showed a general reduction in the parameters studied in relation with the original sperm dose incubated in the same conditions as the backflow was collected. These results suggest that spermatozoa are already subject to a pre-selective process within the uterus before further selection at the utero-tubal junction and in the oviductal isthmus. Our data agree with those of a previous report (Taylor et al., 2008) in which the sperm population was studied in *ex vivo* conditions by the incubation of spermatozoa in different fractions of the uterus. While the binding of viable sperm to the oviduct is thought to act as a sperm reservoir, the retention of sperm cells in the uterus could serve to protect the viable spermatozoa from being removed with the backflow or to help sperm maturation (Taylor et al., 2008), so these findings could be interpreted as a pre-selection process. As far as we know, this is the first time that an analysis of sperm backflow has been reported.

In conclusion, this study shows that post-CAI in field conditions can be totally recommended. This technique can be successfully used in sows instead of CAI and, at the same time, reduce costs. According to our reproductive parameters and our pig production cost analysis, post-CAI 1 is the most profitable technique. However, a full economic study may be necessary depending on the country and farm conditions to clarify which specific post-CAI conditions are the most suitable.

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