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Effect of a GnRH analogue (Maprelin) on the reproductive performance of gilts and sows

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ABSTRACT

The ability of peforelin (l-GnRH-III) to stimulate follicular growth, FSH release, and estrus in gilts after altrenogest treatment and in sows after weaning was investigated. In three farrow-to-wean herds, with at least 600 sows and average production performance, 216 gilts, 335 primiparous, and 1299 pluriparous sows were randomly allocated to three treatments: peforelin (M group: Maprelin), eCG (F group: Folligon), and physiological saline solution (C group). Animals were treated 48 hours after their last altrenogest treatment (gilts) or 24 hours after weaning (sows). The weaning-to-estrus interval, estrus duration, estrus rate (ER), pregnancy rate, and total born (TB), live born, and stillborn (SB) numbers were recorded and compared between treatments for the different parity groups (gilts and primiparous and pluriparous sows). Follicle sizes were measured in representative animals from each group on the occasion of their last altrenogest treatment or at weaning, and also on the occasions of their first (FS1) and second (FS2) attempted inseminations. Blood samples were taken to determine FSH concentrations at weaning and 2 hours after injection, and progesterone concentrations 10 days after the first insemination attempt. The relative change in FSH concentrations was calculated. Significant differences were found for ER within 7 days of weaning in pluriparous sows (95%, 91%, and 90% for the M, F, and C groups, respectively, $P = 0.005$). Gilts in the F-group had high TB numbers, and pluriparous sows in the M group had high SB numbers (TB gilts = 13.6, 15.4, and 14.9 [$P = 0.02$] and SB pluriparous sows = 1.8, 1.4, and 1.7 [$P = 0.05$] for the M, F, and C groups, respectively). The M group had the highest FS1 (for gilts) and FS2 (for pluriparous sows) values: FS1 = 5.4, 4.9, and 4.9 mm [$P = 0.02$] and FS2 = 6.8, 5.3, and 6.3 mm [$P = 0.03$] for the M, F, and C groups, respectively. There were no significant differences between the different treatments within each parity group with respect to any of the other variables. Overall, peforelin treatment had small but positive effects on the ER and follicle growth in certain parity groups but did not seem to affect litter sizes or FSH and progesterone levels in sows on the occasions of the corresponding examinations.

1. Introduction

Maintaining optimal reproductive performance is essential for meeting economic targets in commercial pig production. Management strategies, including accurate feeding at different stages of breeding, batch farrowing,

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optimal housing, and a sufficiently long photoperiod in the insemination facility are not always sufficient to meet farmers' performance requirements. Pharmaceuticals, that is, hormones, can be used to manipulate the estrus cycle in swine, for example, to synchronize estrus and ovulation within a herd, which can increase reproductive performance [1]. In females that have undergone an estrus synchronization program, it is possible to inseminate multiple batches of sows within a short time frame—1 or 2 days—which results in a relatively synchronized onset of farrowing within these batches. These procedures are increasingly important, especially in herds where batch production systems for sows are used or will be used.

Treatment with exogenous gonadotropins in sows after weaning or in gilts after altrenogest treatment has been used to stimulate follicular development and to induce ovulation in prepuberal, cycling, lactating, and anestrus sows [2]. It has also been shown to improve the synchronization of estrus onset within batches [1,3,4]. In addition, gonadotropins have been used to decrease the weaning-to-estrus interval (WEI), which proved to be particularly helpful in sows that were at a high risk of reduced fertility during the post-weaning period, such as first parity sows [5] or animals experiencing seasonal infertility problems [6].

The release of LH and, to a lesser extent, FSH from the pituitary gland is governed by the hypothalamic GnRH [1,2,7,8]. GnRH is therefore a key regulator of the growth, maturation, and, ultimately, the ovulation of follicles. Whereas LH secretion is dependent on GnRH, FSH is not. Instead, the FSH levels are regulated by other peptides, such as gonadal activins, inhibins, and follistatins [8–10]. Twenty years ago, Sower et al. [11] reported for the first time that there is another selective FSH-releasing factor produced by the hypothalamus in fish—specifically, the lamprey, *Petromyzon marinus* (lamprey GnRH-III). This variant of GnRH was put forward as a potential FSH-releasing factor. Numerous subsequent *in vivo* and *in vitro* studies were conducted in different species, yielding inconsistent results. On the basis of *in vitro* and *in vivo* studies with rats, cows, and barrows, treatment with l-GnRH-III induces increases in the levels of FSH but not of LH [10,12–14]. However, studies on mid-luteal intact cows [15] and barrows [16] reported that l-GnRH-III only stimulates the release of LH and does not affect FSH. Still other studies indicated that treatment with 1-GnRH-III did not cause any increase in the levels of either FSH or LH in rodent brain tissues [17] or in gilts [1], but stimulated the secretion of both gonadotropins in rat pituitary cells [18] and ovariectomized cows [15]. To date, no studies have been conducted to explore the influence of l-GnRH-III on the secretion of the different reproductive hormones in gilts and sows at the same time.

Recently, a German company, Veyx, launched the product Maprelin, whose active substance is l-GnRH-III (peforelin). This agent is marketed for the induction of the estrous cycle in sows after weaning and in sexually mature gilts, in animals that have undergone progestogen therapy to inhibit the estrous cycle. Different studies conducted in Germany have suggested that treatment with peforelin (Maprelin, l-GnRH-III, Veyx-Pharma, Schwarzenborn, Germany) has positive effects on estrus induction in gilts and sows [19,20] and reduces the interval between the animals' most recent altrenogest treatment and the onset of estrus in gilts [21,22]. It may also decrease the negative effects of seasonal infertility [19].

The purpose of the study reported herein was to investigate the ability of peforelin to stimulate follicular growth and estrus in gilts after altrenogest treatment and in post-weaning sows, and to study its effects on litter size in Belgian farrow-to-wean herds with average production performance. In addition, FSH and progesterone (P4) levels in the studied animals were analyzed to investigate the effects of l-GnRH-III on FSH release and the ability of the CL to produce P4. The performance of the peforelin-treated animals was compared with that of a pregnant mare serum gonadotropin (ECG)-treated group and an untreated control group.

2. Materials and methods

The study was conducted between January 2010 and May 2011 and was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (approval: EC2010/035).

2.1. Herd selection, study animals, and management practices

Three farrow-to-wean herds in the province of West Flanders with at least 600 sows (600–1700) and an average reproductive performance for the Belgian swine industry were included in the study. Briefly, the number of weaned piglets/sow/year ranged from 23 to 27, and on average, 85% to 95% of the sows reported estrus within 7 days of weaning. More detailed information on the farms is presented in Table 1.

In total, 1945 gilts and sows (average: 650 per herd) were investigated during one reproductive cycle, starting at the point of weaning for sows or from their most recent altrenogest treatment for gilts, to their subsequent weaning (Table 1). Animals with clinical disease

Table 1
Characteristics of the three pig herds included in the study.

Characteristics	Herd A	Herd B	Herd C
Number of sows per herd	1200	1700	600
Number of sows included in study	627	685	633
Breed of sows	Danbred × York	PIC	Topigs20
Batch-production system for sows (weeks)	1	2	4
Lactation period (weeks)	3	3	3
Piglets weaned/sow/y	25.9	26.1	26.3
Average weaning-to-insemination interval (days)	7.0	7.1	7.8
Age of gilts at first insemination (days)	280	290	250

and/or reproductive disorders, such as puerperal disease or pathological vaginal discharge, were not included. Gilts had been treated with altrenogest (Regumate, MSD Animal Health, Brussels, Belgium) for 18 days (20 mg per gilt per day, administered orally) after having shown at least one estrus. To ensure accurate dosing, gilts were housed in individual stalls during altrenogest treatment. Sows were weaned on Days 20 to 21 of lactation. One day after the final altrenogest treatment (gilts) or weaning (sows), the animals were moved to a breeding facility, with individual housing and a light schedule of 16 hours per day giving 250 lux, measured at the sows' heads.

Estrus stimulation started on the first day post-weaning (pw) in sows or 48 hours after the last altrenogest treatment in gilts (for the sake of convenience and consistency, the day of the last altrenogest treatment is henceforth referred to as the first day post-weaning or "pw"), using at least two teaser boars. All animals were fed *ad libitum* with a gestation feed from Day 1 pw until insemination. A supplement of 150 mg dextrose per day per animal was provided as a top dressing. To further optimize estrus stimulation and detection, supplemental boar noises were played to the animals in herd A via a voice recorder, and herd C used a Contact-O-Max (Ro-Main Europe, France), which is a remote-controlled mobile unit with a boar inside.

Estrus detection was performed twice a day (am and pm) from Day 4 pw onwards. The same artificial insemination (AI) schedule was used in all three herds. Briefly, sows showing standing estrus on Day 4 pw in the morning were inseminated 24 hours later, and those showing estrus in the evening were inseminated 12 hours later. Sows showing standing estrus on Day 5 were inseminated 8 hours later, whereas those showing estrus on Day 6 pw were inseminated immediately. Sows that still showed estrus 12 hours after their first round of AI were inseminated a second time, and a third time in the rare cases where standing estrus persisted for 24 hours. Single sire semen from boars of proven fertility was purchased from a commercial AI center.

Pregnancy testing was performed by the herd veterinarian using ultrasound at 23 to 28 days of gestation and again 2 weeks later. Gilts and sows that were found to be pregnant at 23 to 28 days were moved to the gestation unit. In herds A and B, pregnant females were housed in groups, with the exception of gilts and sows that had previously experienced reproductive problems (e.g., repeat breeding) in herd A. In herd C, only gilts were housed in groups, and weaned sows were housed in individual stalls. In all three herds, animals were fed a gestation diet *ad libitum* in the group housing-gestation unit.

All of the participating herds used similar vaccination schedules for their sows. Sows were vaccinated for Parvovirus and *Erysipelothrix rhusiopathiae* (2 weeks *postpartum*), *Escherichia coli* (2 weeks *prepartum* in herd A), atrophic rhinitis (2 weeks *prepartum* in herds A and C), Porcine Respiratory and Reproductive Syndrome virus (four times a year in herds B and C), and, finally, Swine Influenza Virus (three times a year in herd C).

2.2. Experimental design

The study population was grouped into three age categories: gilts and primiparous and pluriparous sows. Within each age category, animals were randomly allocated to one of three treatment groups before treatment: peforelin (the M group), in which gilts and pluriparous sows were treated with 150 μg peforelin, corresponding to 2 mL of Maprelin based on the manufacturers' documentation, and primiparous sows were treated with 37.5 μg peforelin, corresponding to 0.5 mL of Maprelin; equine Chorion Gonadotropin (eCG; the F group) as a positive control, in which animals were treated with 1000 IU eCG, corresponding to 1 mL of Folligon, MSD Animal Health, Brussels, Belgium; and physiological saline solution as a negative control (the C group), in which animals were treated with 1 mL of physiologic saline solution.

All treatments were applied via intramuscular injection into the neck 24(±1) hours pw (sows) or 48(±1) hours after their last altrenogest treatment (gilts). The entire study, including estrus detection, AI, and the recording of the different parameters, was conducted using a blinded design.

2.3. Major parameters

2.3.1. Estrus and pregnancy

The measured variables were the estrus rate (ER, the proportion of gilts and sows showing estrus), WEI (the interval between the day of the last altrenogest treatment for gilts or the day of weaning for sows and the onset of estrus), estrus duration (ED, the interval between the detection of the first and last standing estrus), and pregnancy rate (PR, the proportion of pregnant animals from the animals inseminated).

2.3.2. Litter size

The number of total born (TB), live born (LB), stillborn (SB), and mummified (M) piglets was recorded for each litter.

2.4. Minor parameters

For the minor parameters, 10 animals per age group and per treatment group in each herd (i.e., 90 animals per herd) were selected at random and individually identified at weaning, that is, 24 hours before treatment for sows or on the last day of altrenogest treatment for gilts.

2.4.1. Follicle size

Ovary scanning was conducted according to the procedures described earlier [23]. The ovaries of the sows were monitored using transabdominal ultrasound scans performed with a sectorial probe (5 MHz, MS Multiscan digital, MS Schippers, The Netherlands) to estimate the average follicle size. Ultrasound scans were performed twice daily, at intervals of ~8 hours, at the time of weaning or the last altrenogest treatment (FS0), and during the first (FS1) and second (FS2) insemination attempts.

The latter two scans were conducted to estimate whether ovulation had already occurred, that is, to detect the presence of follicles with diameters in excess of 2 mm following larger follicles, as well as to identify potential abnormalities such as ovarian cysts, that is, cyst-like formations with diameters of >15 mm. Where possible, four (at minimum, two) clearly defined follicles were measured in the right ovary, after which the mean follicle size was calculated to assess the follicular diameter. Ultrasound testing was always performed by the same experienced person (first author).

2.4.2. FSH and P4 concentrations

Three blood samples for hormone analyses were drawn by venopuncture from the "vena jugularis." Samples were collected immediately before treatment in order to determine a baseline concentration of FSH; 2 hours (±0.5) after treatment, in order to determine the effect of l-GnRH-III on the release of FSH, and, finally, on the 10th day after the first AI attempt, to determine the capability of the CL to produce P4. Samples were transported to the Faculty of Veterinary Medicine (Ghent University, Merelbeke, Belgium) and centrifuged for 10 minutes at 2.504 × g at 4 °C within 12 hours of collection. The serum was then collected and stored at −20 °C until analysis.

For analysis, the serum was shipped in bulk to the laboratory of the Faculty of Veterinary Medicine in Leipzig (Germany). FSH concentrations were determined in the first two blood samples by RIA following the procedure described by Kauffold et al. [24]. The limit of detection was 0.4 ng/mL, and the intra- and interassay coefficients of variation (CVs) were 6.4% and 10.6%, respectively. P4 analysis was performed using the third samples (i.e., those collected 10 days after the first AI attempt), as described by Brüssow et al. [1]. The intra- and interassay CVs for this procedure were 7.5% and 8.1% respectively, and its lower limit of detection was 0.5 ng/mL.

The mean FSH levels before treatment for females within the treatment and parity groups were used as baseline concentrations, and the relative change in concentration between the post- and pretreatment periods was used as the treatment response.

2.5. Statistical analysis

The number of animals in each age category (gilts, primiparous, and pluriparous) was sufficient to detect differences of at least 5.0% in the ERs between the groups with 95% confidence, 80% power, and a standard deviation of 3.1 (WinEpiscope 2.0) [25]. Data analysis was conducted in a blinded manner. All statistical calculations were performed using version 20.0 of the SPSS software package (SPSS Inc., Chicago, IL, USA).

The normality of the data sets was tested using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The results for the different treatment groups were expressed as arithmetic means and the corresponding SDs. For all parameters, separate analyses were performed for animals with a WEI ≤7 days after weaning and those with a WEI of >7 days. Comparisons between the three treatment groups were made for all animals and separately for the three different parity groups. For all parameters, the effect of parity and herd was significant. Therefore, three different analyses were performed per parity group and herd was included in the statistical model. Multiple comparisons for the parameters TB, LB, SB, M, and FS were performed using ANOVA. Pairwise comparisons between groups were conducted using the *post hoc* Bonferroni test. For parameters with non-normal distributions (WEI, ED, FSH, and P4), nonparametric tests were used. Cross-tabulations and the Chi-squared test were used to detect differences between the treatment groups with respect to the ER and PR parameters. The significance threshold applied was $P \leq 0.05$.

3. Results

Results for a total of 1918 animals were included in the statistical analysis. Twenty-seven sows (1.4%) had incomplete records and were excluded from the analysis.

3.1. Major parameters

3.1.1. Estrus and pregnancy

The ER ≤7 days and the WEI ≤7 days for the three different treatment and parity groups are shown in Table 2.

For pluriparous sows, the ER ≤7 days were significantly ($P = 0.005$) higher in the M group (95%) than the F (91%) or the C group (90%).

The WEI ≤7 days tended to be shorter in the F group (4.5 days) than the C or M groups (4.7 days) in primiparous sows ($P = 0.07$). The WEI >7 days in gilts was greater than 21 days in the M and C groups ($P = 0.05$) and also in the M and F groups for pluriparous sows ($P = 0.07$). For primiparous sows, the WEI >7 days value was greater than 21 days in all the three treatment groups. There were no significant differences between any of the treatments for each parity group with respect to their ED ≤7-day values (mean = 36.3 ± 16.0, 39.6 ± 14.1, and 43.0 ± 14.9 hours for gilts, primiparous, and pluriparous sows, respectively) nor with respect to their PR ≤7-day values (mean = 82%, 79%, 84% for gilts, primiparous, and pluriparous sows, respectively).

Table 2
ER and WEI for the different treatment (M = Maprelin, F = Folligon, C = control) and parity groups, in estrus within 7 days of weaning (≤7 d; SD).

Parity	Group	*n*	ER ≤7 d (%)	WEI ≤7 d ± SD (d)
Gilts	M	83	73	5.3 ± 1.0
	F	73	71	5.7 ± 1.0
	C	77	74	5.6 ± 1.0
Primiparous	M	129	88	4.7[c] ± 0.8
	F	109	90	4.5[d] ± 0.9
	C	108	90	4.7[c] ± 0.8
Pluriparous	M	446	95[a]	4.5 ± 0.8
	F	432	91[b]	4.5 ± 0.8
	C	461	90[b]	4.5 ± 0.8

[a,b] Within a specific parity group, differences between treatment groups were statistically significant ($P \leq 0.05$).
[c,d] Within a specific parity group, differences between treatment groups showed a tendency ($P = 0.07$).

3.1.2. Litter size

Table 3 presents the TB, LB, and SB numbers for the different parity and treatment groups.

The TB number was significantly higher in the F group (15.4 piglets) than the M group (13.6 piglets) in gilts (P = 0.02). In primiparous sows, the TB number for the F group tended to be greater than in the C group (15.4 vs. 14.1, respectively, P = 0.09).

The SB number was higher in the M group (1.8 piglets) than in the F group (1.4 piglets) in pluriparous sows (P = 0.05).

The number of M per litter was similar for all treatment groups and for all parity groups (0.2 ± 0.5 mummies, P > 0.05).

3.2. Minor parameters

3.2.1. Follicle size

The percentage of sows that had no follicles with diameters above 2 mm at weaning and still had only small follicles on their first and second AI attempts (i.e., those that experienced no post-weaning follicular growth or had already ovulated) was 0%, 8%, and 23%, respectively, over all animals and all treatments. There were no significant differences between the treatment groups with respect to these variables. Polycystic ovaries were found in three sows at first AI, one from the F group and two from the C group. These sows were excluded from subsequent analyses.

The FS0, FS1, and FS2 results are presented in Table 4. The mean FS1 value was significantly larger in the M group (5.4 mm) than the F (4.9 mm) or C groups (4.9 mm) for gilts (P = 0.02). The mean FS2 value was significantly larger in the M group (6.8 mm) than the F group (5.3 mm) in pluriparous sows (P = 0.03).

3.2.2. FSH and P4 concentrations

There was no significant difference between the treatment groups with respect to the relative change in mean FSH levels over the studied period (−0.04 ± 0.43, 0.19 ± 1.09, and 0.04 ± 0.66 μg/L for gilts and primiparous and pluriparous sows, respectively). There was no increase in FSH levels following treatment in either of the treatment groups.

Table 3
The number of TB, LB, and SB piglets for the different treatment (M = Maprelin, F = Folligon, C = control) and parity groups (SD).

Parity	Group	*n*	TB ± SD	LB ± SD	SB ± SD
Gilts	M	49	13.6 ± 3.5[b]	12.8 ± 3.2	0.7 ± 1.1
	F	42	15.4 ± 2.4[a]	14.2 ± 2.5	1.0 ± 1.3
	C	48	14.9 ± 2.9[a,b]	13.9 ± 3.6	0.9 ± 1.9
Primiparous	M	90	14.7 ± 3.6[c,d]	13.5 ± 3.7	1.1 ± 2.0
	F	76	15.4 ± 3.6[d]	14.1 ± 3.6	1.3 ± 2.1
	C	74	14.1 ± 3.3[c]	12.9 ± 3.7	1.0 ± 1.5
Pluriparous	M	347	15.4 ± 3.5	13.5 ± 3.3	1.8 ± 2.0[b]
	F	332	14.8 ± 3.9	13.2 ± 3.5	1.4 ± 1.9[a]
	C	341	15.0 ± 3.9	13.2 ± 3.6	1.7 ± 2.2[a,b]

[a,b] Within a specific parity group, differences between treatment groups were statistically significant (P ≤ 0.05).
[c,d] Within a specific parity group, differences between treatment groups showed a tendency (P = 0.09).

Table 4
Follicle size (mean ± SD; in mm) at weaning (FS0) and at first (FS1) and second insemination (FS2) for the different treatment (M = Maprelin, F = Folligon, C = control) and parity groups.

Parity	Group	*n*	FS0 ± SD	FS1 ± SD	FS2 ± SD
Gilts	M	40	2.6 ± 0.8	5.4[b] ± 1.0	5.7 ± 1.8
	F	20	2.5 ± 0.8	4.9[a] ± 0.6	5.0 ± 1.7
	C	32	2.5 ± 1.1	4.9[a] ± 1.2	5.9 ± 1.7
Primiparous	M	35	2.9 ± 0.8	5.5 ± 1.1	6.2 ± 2.0
	F	23	2.8 ± 1.4	5.7 ± 1.8	6.2 ± 1.9
	C	30	3.1 ± 1.0	5.6 ± 1.3	5.8 ± 2.5
Pluriparous	M	27	3.0 ± 1.1	5.5 ± 1.3	6.8[a] ± 2.3
	F	34	3.1 ± 1.1	5.6 ± 1.6	5.3[b] ± 2.5
	C	34	3.4 ± 1.1	5.4 ± 1.3	6.3[a,b] ± 2.3

[a,b] Within a specific parity group, differences between treatment groups were statistically significant (P ≤ 0.05).

The mean P4 levels in the F group (15.24 ng/mL) tended to be lower than those in the M (17.86 ng/mL) and C (20.50 ng/mL) groups for primiparous sows (P = 0.07). No significant differences were observed in either gilts or pluriparous sows (19.95 ± 6.43 and 17.97 ± 5.68 ng/mL for gilts and pluriparous sows, respectively).

4. Discussion

This study was conducted to determine the effects of peforelin, that is, synthetic l-GnRH-III, on the reproductive capabilities of gilts after altrenogest treatment and post-weaning sows in commercial Belgian pig herds. All herds had an average to suboptimal reproductive performance on the basis of recent benchmarking data for Belgian and Dutch farms (PR = 88%, WEI = 5.6, and weaned piglets/sows/year = 28.5, Agrovision Herd monitoring 2011, Cerco Soft N.V., Oudenaarde, Belgium). In general, the differences between the treatment groups were relatively small for all of the studied variables. Statistically significant differences were only observed for the ER in pluriparous sows, the follicle size at AI for gilts and pluriparous sows, and the total numbers of born and SB piglets in gilts and pluriparous sows, respectively.

Significantly more pluriparous sows in the peforelin treatment group reported estrus within 7 days of weaning than was the case for the negative control group or the eCG treatment group. This is important from an economical and practical perspective because it reduces the number of nonproductive days. Assuming a cost of €3.5 per sow per nonproductive day [26] and an average treatment cost of €3.2 per treated sow for peforelin, the elimination of even one nonproductive day would be economically beneficial (€0.3 profit per sow per day). Because peforelin treatment increased the number of sows in estrus within 7 days of weaning by 5% in herds with 650 sows on average, it would save the farmer almost €10 per day (32.5 sows * €0.3). Peforelin treatment can also be easily incorporated into sow batch management systems. Sows that do not enter estrus within a set time frame in a batch production system are good candidates for culling, but are frequently given another chance in order to limit the replacement rate. However, if the proportion of sows that do not enter estrus can be decreased sufficiently, as was the case for

pluriparous sows treated with peforelin, these problematic sows can safely be culled and replaced. It is not clear why this effect was only seen in pluriparous sows. Engl et al. [19,20,22] observed an increase in ER for all parity groups treated with peforelin (relative to eCG treatment). It is worth mentioning that according to the participating producers, the gilts treated with peforelin had the best performance in terms of ER (personal communication). However, the measured ER data do not support this observation. The physical body condition of the sows is very important for the reproductive cycle [27,28], and major back fat losses during lactation may negatively influence the outcome of their estrous performance. However, it is unlikely that differences in metabolic stage alone can explain the aforementioned discrepancies in the ER data, because there were no differences in back fat loss between the studied groups (data not shown).

There were no differences between the treatment groups with respect to ED and WEI, with one exception: primiparous sows treated with eCG tended to have shorter WEI values. This is consistent with the results of Engl [21] and Engl et al. [20], and may occur because eCG exhibits both LH- and FSH-like activities [29]; LH stimulates the growth of follicles from 4 mm to preovulatory size [30], which in turn shortens the follicular phase and thus the WEI [31].

The PR of the sows examined in this work was ~80% and was lower than the PR obtained before the study (±85%). This may indicate that the selected herds did not have optimal reproductive performance, because the typical PR target values are 90% or more [32]. The reason for the lower PR in this case is not clear. However, in 23% of the studied sows, no follicles were seen at the second AI, indicating that they had already ovulated. Therefore, it is possible that the timing of the insemination was not optimal in (some of) these sows [33] and that the relatively low PR values in the study were due to the use of an inappropriate insemination scheme.

The lack of significant differences with respect to TB between the control and treatment groups could indicate the safety of the products, because they did not induce superovulation. This would be consistent with the results of Manjarin et al. [34] and Patterson et al. [5]. More piglets were born to gilts and primiparous sows treated with PMSG than to untreated animals or animals injected with peforelin. According to Brüssow and Wähner [35], PMSG is the only agent that can stimulate sufficient ovulatory follicles to produce large viable litters. However, do Lago et al. [36] and Martinat-Botté et al. [3] found that PMSG treatment increased the ovulation rate, but also had a negative influence on embryonic viability, probably because it increased follicular heterogeneity in the preovulatory pool and caused the asynchronous development of embryos [37,38].

Previous studies [10,12–14] have shown that l-GnRH-III treatment increases FSH levels. Increased levels of FSH during the follicular phase increase follicular size [39,40] and the size of the CL [38,41], which lead to elevated P4 levels [42].

The largest follicles at insemination were observed in gilts and pluriparous sows treated with peforelin. This is in keeping with the results of Engl [21], who suggested that peforelin promotes the release of FSH [10,12–14]. Surprisingly, FSH levels did not increase significantly following treatment in any group examined in this work, including the peforelin group. It is possible that the animals' FSH levels increased rapidly after treatment but then returned to the baseline level within 2 hours of injection. This would be consistent with the report of Kauffold et al. [10], who observed that FSH levels peaked at 205% of their initial value 1 hour after peforelin treatment in barrows. Dees et al. [14] found that the peak response occurred within 15 minutes of treatment and that basal FSH levels were restored 1.5 hours after stimulation with l-GnRH-III in cows. The results obtained in this work are consistent with those reported by Brüssow et al. [1] and Barretero-Hernandez et al. [16], who used l-GnRH-III in either gilts or barrows and found no evidence of FSH-releasing activity. It is therefore not clear why peforelin-treated animals had larger follicles than those seen in other treatment groups at first AI, nor can the results of this study explain the differences between the results of previous studies with respect to the FSH-releasing activity of l-GnRH-III. It may be that l-GnRH-III acts locally at the ovarian level, as has been shown for GnRH in rats [43].

Wientjes et al. [42] reported that there is a positive relationship between follicle size and the size and weight of the CL, indicating that larger follicles develop into larger CL, which then produce more P4. Although treatment with peforelin increased follicle diameter in this work, this did not significantly increase P4 levels. Suboptimal LH surge levels could potentially cause inadequate luteinization of the ovulated follicles and therefore reduce plasma P4 levels and increasing embryo mortality [44]. Because LH was not measured in this work, no conclusion can be drawn on this matter. However, the P4 levels in pregnant sows were significantly higher than in their nonpregnant counterparts ($P = 0.03$, data not shown), indicating that the timing of blood sampling was correct and that the lack of differences between groups with respect to their P4 levels was not due to inappropriate sampling.

The trial was conducted in three different herds, with similar reproductive histories. The management procedures applied to the three herds were all relatively similar in terms of weaning, insemination, housing, and feeding regimes. In addition, seasonal effects can be ruled out because the study was conducted over a period of 17 months. Nevertheless, a significant herd effect was observed for all of the studied parameters; this may have been related to the breed of the sows. The study was conducted in a double-blinded fashion because estrus detection was performed by the farmers who were blinded to the applied treatments, and the statistical analysis was performed by an independent statistician.

5. Conclusion

The results presented herein indicate that treatment with peforelin caused a significant increase in the number of pluriparous sows in estrus within 7 days of weaning. Peforelin also seems to have a positive effect on follicle growth in gilts and pluriparous sows. If the number of

sows that have not entered estrus within 7 days can be minimized, for example, by treatment with peforelin, culling decisions become easier to make and losses due to nonproductive days are minimized, which can save farmers up to €10 per day. However, the administration of hormonal products cannot be used as a substitute for adequate management.

Further studies on the FSH-releasing activity of l-GnRH-III are warranted because the available data on this topic are highly inconsistent; in almost half of the previous studies, there was no increase in FSH levels following 1-GnRH-III treatment.

The influence of peforelin on the birth weight of the piglets produced by gilts and sows and the performance of their subsequent litters will be described in a second paper.

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