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A novel semen supplement (SuinFort) improves sow fertility after artificial insemination

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ABSTRACT

The objective of this study was to determine effects of a novel seminal supplement, SuinFort, on fertility of multiparous sows. For 2 years, a total of 1159 sows were assigned to be artificial inseminated (AI) either with semen supplemented with the additive (2 IU oxytocin, 5 μg lecirelin, 2 mM caffeine, $n = 830$ AI) or with no supplementation (Control, $n = 2422$ AI). The supplement was included 15 min before insemination. Supplementation with SuinFort resulted in greater fertility both by increasing farrowing rate 87.2% ± 0.7 to 90.7% ± 1.0 ($P < 0.001$) and litter size from 13.8 ± 0.1 to 14.4 ± 0.1 ($P < 0.001$). To test if there was a direct effect of SuinFort on spermatozoa, an *in vitro* experiment was conducted using semen doses from 10 boars. Semen was stored at 15 °C and on days 1, 2 and 3 were aliquoted to a control and SuinFort-supplemented group, incubated at 37 °C and analyzed for sperm quality at 15 min and 2 h. For aliquots with SuinFort, there was a small decrease in semen quality. In conclusion, the administration of a combination of oxytocin, lecirelin and caffeine to boar semen 15 min prior to AI, positively affects sow fertility. Considering that *in vitro* effects on sperm quality were small, it is likely that SuinFort affects fertility by modulating uterine function. Supplementation of semen with SuinFort, therefore, has potential for increasing pork production efficiency as a result of increased reproductive efficiency after AI of sows.

1. Introduction

Maximizing reproductive output during the sow's lifetime decreases production costs in commercial breeding herds. Use of artificial insemination (AI) can improve reproductive efficiency as compared with natural mating but can be associated with reduced sow reproductive performance (Knox, 2014). Inadequate reproductive tract stimulation of the sow during AI may compromise myometrial contractions, impairing sperm transport to the oviduct sperm reservoir that is essential for fertilization, thus, potentially reducing subsequent fertilization rate and litter size (Claus, 1990).

During natural breeding, the presence of the boar induces oxytocin release in the sow increasing uterine activity (Claus and

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Schams, 1990; Langendijk et al., 2003). Also, estrogens in boar seminal plasma increase myometrial contractions by causing the local release of prostaglandin (PG) F2α from the endometrium (Claus et al., 1990). Furthermore, seminal estrogens can enter the circulation affecting endogenous gonadotropin release and time of ovulation (Claus et al., 1990; Waberski, 1997). With the usual semen processing procedures, estrogen content of seminal doses for AI is markedly less than the amount of estrogen in unprocessed semen (Kirkwood and Thacker, 1991). Injection or vaginal deposition of GnRH can also alter ovulation timing in relation to behavioral estrous expression (Kirkwood and Kauffold, 2015). Interestingly, improvements in farrowing rate and litter size have been reported following the addition of estrogen (Claus et al., 1989), PGF2α (Peña et al., 2000) or oxytocin (Duziński et al., 2014) to the seminal dose.

A further potential seminal additive is methylxanthine (caffeine). Adequate progressive sperm motility is essential for the establishment of an optimal sperm reservoir in the oviduct, and the addition of caffeine to boar sperm increases cell motility (Miyamoto and Nishikawa, 1980; Bury et al., 2017). Supplementation of boar with caffeine could also benefit fertility by increasing capacitation and related spermatozoa processes (Yamaguchi et al., 2009). In addition to the potential direct effect of caffeine on spermatozoa, supplementation with caffeine could also improve reproductive efficiency after AI by modulating the uterine environment. Caffeine, at least in larger amounts, functions as an immunomodulator, reducing the function of some immune cells and the production of some cytokines and receptors (Al Reef and Ghanem, 2018). Indeed, results from *in vitro* experiments indicate that supplementation with caffeine reduced phagocytosis and chemotaxis of neutrophils of spermatozoa (Li and Funahashi, 2010; Li et al., 2011). There are reports where there is an improvement of reproductive results in gilts and sows when there is use of AI (Yamaguchi et al., 2009, 2013). These improvements in reproduction with use of AI compared with natural mating have been attributed to there being larger numbers of sperm in the uterus as a result of reduction of the intra-uterine inflammatory reaction with use of AI as compared with natural mating (Matthijs et al., 2003; Yamaguchi et al., 2013).

In the present study the hypothesis, therefore, was that inclusion of a semen supplementation of oxytocin, GnRH and caffeine (i.e., SuinFort) would enhance sow fertility. The hypothesis was tested in a field experiment with examination of both sow fertility and prolificacy. Additionally, to test if the results with SuinFort supplementation could be due to a direct effect on sperm physiology, an *in vitro* test was conducted with liquid-stored boar spermatozoa. The supplementation of boar semen diluent with SuinFort could help improve efficiency of pork production as a result of associated economic benefits.

2. Materials and methods

2.1. Animals and treatments

The University of León Animal Care Committee reviewed and approved the protocol and procedures. The study was conducted on a 800-sow farrow-to-finish facility near Burgos, Spain in a temperature-controlled building at 18 to 24 °C (cooling panels and thermostats) and a constant 12-h photoperiod. Sows (Landrace × Large White) were housed individually in farrowing crates from 1 week before farrowing until weaning at 21 d, then in individual gestation stalls until confirmed pregnant at 35 d after AI. Pregnant sows were then housed in pens with 8 to 10 sows/pen. Farrowing room temperatures were maintained between 18 and 24 °C throughout the year with a minimum daily photoperiod of 12 h with the use of coolers and artificial lighting.

The semen for AI was obtained from ten Landrace and Large White boars (12–24 months of age) housed in the Technological Centre of Artificial Insemination (León, Spain) and routinely used for the production of semen doses for AI. Semen was evaluated for quality and only ejaculates with at least 75% motile and 70% morphologically normal spermatozoa were used. The semen (sperm-rich fraction) was collected using the gloved-hand method into a pre-warmed container with 100 ml of MR-A® extender (Kubus, Madrid, Spain), assessed for quality and processed for producing 80-mL doses with 3×10^9 spermatozoa. Doses were stored at 15 °C until used within 48 h of the time it was collected for AI.

2.2. Insemination experiments

During 2 years, the fertility of 1159 multiparous Landrace × Large White sows was examined in response to supplementation with SuinFort (for a total of 3252 inseminations). At estrus detection (boar presence and response to back pressure), sows were randomly assigned for insemination with semen doses containing 2 IU oxytocin, 5 µg GnRH analog (lecirelin), and 2 mM caffeine (n = 830; SuinFort®, Patent ES2608935), or served as non-supplemented controls (n = 2422). The dose of oxytocin was selected based on its efficacy in previous studies (Peña et al., 2000; Duziński et al., 2014). For the lecirelin dose, it was assumed that 1.0 µg of lecirelin has similar activity as 50 µg of PGF2α. SuinFort was added to the first seminal dose 15 min before insemination (Peláez et al., 2006). All inseminations were performed by the same operator and reproductive results were collected at the time of parturition to allow for the determination of farrowing rates and subsequent litter sizes.

2.3. In vitro test on sperm quality

Sperm doses from ten Large **White** boars (from the same center but different from those used in the AI trial) were stored at 15 °C for 3 days with semen evaluations being performed each day during the storage period. The semen was warmed to 25 °C and aliquoted into two 1-mL samples. One aliquot was supplemented with 10 µl of the additive, and adding 10 µl of MR-A® to the control sample. Both samples were incubated at 37 °C and sperm quality was assessed 15 min after adding SuinFort and again after 2 h of storage.

Motility was assessed using CASA (Fernández-Gago et al., 2017). A 5 µl droplet of sample was placed in a Makler chamber (10 µm depth; Haifa Instruments, Israel) and observed using a phase contrast microscope (Nikon E400; Nikon, Tokio, Japan) with a warmed stage at 37 °C and 10× negative contrast optics). At least five fields and 200 cells were recorded from 53 frames/s for 1 s using a Basler A302fs digital camera (Basler Vision Technologies, Ahrensburg, Germany). The images were processed using ISAS software v. 1.19 (Proiser, Valencia, Spain), with 25 to 80 µm for head area and a VCL (curvilinear path velocity) > 10 µm/s to classify a spermatozoon as being motile. The following variables were evaluated in raw semen: total motility (MOT), progressive motility (PROG, VAP > 25 µm/s and STR > 60%), VCL, VSL (straight path velocity), VAP (average path velocity according to the average smoothed path; µm/s), LIN (linearity), STR (straightness), WOB (wobble), ALH (amplitude of the lateral displacement of the sperm head), and BCF (frequency of the flagellar beat).

Flow cytometry analyses (Fernández-Gago et al., 2013) were conducted using a CyAn ADP (Beckman Coulter; Brea, CA), equipped with three diode lasers **(violet** at 405 nm, blue at 488 nm and red at 635 nm). Samples were added to 300 µl of PBS with 0.5% BSA at 10^6/ml and stained with different probes (ThermoFisher; Waltham, MA) for 15 min at 37 °C, with final concentration: Hoechst 33342 (H342, 4.5 µM) for discriminating debris; propidium iodide (PI; 1.5 µM) for viability; YO-PRO-1 (100 nM) for apoptotic-like changes; PNA-FITC (PNA; 1 µg/ml) for assessing the acrosomal status; merocyanine 540 (MC; 2 µM) for assessing capacitation-associated changes; MitoTracker Deep red (MT; 100 nM) for assessing mitochondrial activity. These probes were combined as H342/YO-PRO-1/M540/PI/MT for assessing viability, apoptosis, capacitation, and mitochondrial activity as well as H342/PNA/PI for assessing viability and acrosomal status. The fluorescence was collected using photodetectors with filters 450/50 **(violet** line, blue fluorescence: H342), 530/40 (blue line, green fluorescence: YO-PRO-1, PNA-FITC), 575/25 (blue line, **orange** fluorescence: Merocyanine 540), 613/20 (blue line, red fluorescence: PI) and 665/20 (red line, red fluorescence: MT). At least, 5000 spermatozoa were collected per sample, with a flow rate of 200 cells/s. The analysis of the flow cytometry data was conducted using Weasel v. 3.4 (WEHI, Melbourne, Australia).

2.4. Statistical analysis

Differences in farrowing rates were examined using logistic regression analysis utilizing a generalized linear mixed-effects model of the R statistics program (v. 3.5.1). In the case of prolificacy (total, live and dead born piglets), it was assumed there was a Poisson distribution. The linear models included treatment, period and the interaction as fixed effects and sow as well as boar (semen dose) as random effects. For the analysis of the *in vitro* experimental data, there was use of models with storage day, hour and treatment as fixed effects and the boar in the random part of the models. Results are provided as means ± SEM. Effects were considered significant at $P \leq 0.05$.

3. Results

The SuinFort supplementation resulted in greater ($P < 0.001$) fertility as a result of enhanced farrowing rates (Table 1) and prolificacy (Table 2). There was no effect of season nor a was there a SuinFort supplementation × season interaction. The number of stillborn piglets was not affected by SuinFort supplementation.

The results of the *in vitro* experiment indicated the supplementation of SuinFort had a small effect, as compared with that of the Control, on sperm quality, mostly by reducing sperm motility and viability. Sperm motility (Table 3) was slightly less with SuinFort supplementation of semen diluent than that of the control sample. There was an overall effect on total sperm motility and velocity (VAP) with values being -6.9 ± 2.5 ($P = 0.008$) and -4.1 ± 1.1 ($P < 0.001$). Sperm progressive motility and ALH were affected by the SuinFort supplementation only in some day × incubation combinations. Interestingly, the SuinFort supplementation had a positive effect on sperm linearity at 15 min (except on day 3 with $P = 0.091$) after addition of the SuinFort supplement. Furthermore, there was a greater LIN after incubation for control and SuinFort-supplemented samples, but there was a greater increment of the increase for the control samples (significant interaction $P < 0.001$), resulting in the controls having greater LIN after incubation ($P < 0.001$). The results using flow cytometry (Table 4) indicated the SuinFort-supplementation effect on sperm viability and mitochondrial activity was less than that of the control samples (overall, -9.6 ± 3.8 and -6.1 ± 2.4, respectively, $P = 0.016$). Sperm

Table 1
Effect of the SuinFort seminal supplement[†] and season on sow fertility; Numbers are farrowing/total AI, with proportion (%) and SEM within brackets; No effects of season or interaction supplement × season.

	Control	SuinFort
Spring	593/672 (88.2 ± 1.2)	186/200 (93.0 ± 1.8)
Summer	508/592 (85.8 ± 1.4)	243/269 (90.3 ± 1.8)
Autumn	432/499 (86.6 ± 1.5)	185/206 (89.8 ± 2.1)
Winter	573/659 (86.9 ± 1.3)	139/155 (89.7 ± 2.4)
Overall	2106/2422 (87.0 ± 0.7)[a]	753/830 (90.7 ± 1.0)[b]

[a,b]Different superscripts indicate differences $P < 0.001$ for the supplement as main effect.
[†] SuinFort: 2 IU oxytocin + 0.5 µg lecirelin + 2 mM caffeine.

Table 2
Effect of the SuinFort seminal supplementation[†] and season on sow prolificacy; Numbers are piglets born, mean ± SEM of total, alive and dead; No effect of interaction of supplement × season.

	Total		Alive		Dead	
	Control	SuinFort	Control	SuinFort	Control	SuinFort
Spring	13.7 ± 0.1	14.5 ± 0.2	12.8 ± 0.1	12.8 ± 0.2	1.5 ± 0.1	1.7 ± 0.1
Summer	13.9 ± 0.1	14.4 ± 0.1	12.3 ± 0.1	12.9 ± 0.2	1.6 ± 0.1	1.5 ± 0.1
Autumn	13.8 ± 0.1	14.2 ± 0.2	12.4 ± 0.1	12.7 ± 0.2	1.5 ± 0.1	1.9 ± 0.1
Winter	13.7 ± 0.1	14.5 ± 0.2	12.4 ± 0.1	13.2 ± 0.2	1.9 ± 0.1	1.4 ± 0.1
Overall	13.8 ± 0.1[a]	14.4 ± 0.1[b]	12.3 ± 0.1[a]	12.9 ± 0.1[b]	1.5 ± 0.0	1.5 ± 0.1

[a,b]Different superscripts indicate differences $P < 0.001$ for the supplement as main effect.
[†] SuinFort: 2 IU oxytocin + 0.5 µg lecirelin + 2 mM caffeine.

Table 3
Sperm motility results (CASA variables) after incubating refrigerated-stored boar semen (1, 2 or 3 days) with the SuinFort supplement (+) or no supplement (-) at 37 °C Results are mean ± SEM.

Day	Incubation	SuinFort	MOT[†‡] (%)	PROG[‡] (%)	VAP[†] (µm/s)	LIN (%)	ALH (µm)
1	15 min	–	74.7 ± 4.3	10.9 ± 2.9	42.8 ± 2.8	32.2 ± 3.8[a]	1.9 ± 0.2[a]
		+	67.4 ± 3.1	14.2 ± 2.5	36.0 ± 1.5	41.4 ± 4.1[b]	1.5 ± 0.1[b]
	2 h	–	60.8 ± 2.9	28.6 ± 3.1	35.5 ± 2.6	59.2 ± 2.9[a]	1.3 ± 0.1
		+	50.5 ± 3.5	18.6 ± 3.4	31.7 ± 2.0	45.4 ± 5.8[b]	1.3 ± 0.1
2	15 min	–	65.7 ± 3.7	8.7 ± 2.2[a]	40.9 ± 2.1	33.2 ± 2.7[a]	1.6 ± 0.1
		+	61.3 ± 4.0	11.8 ± 2.4[b]	38.2 ± 1.7	38.8 ± 3.1[b]	1.5 ± 0.1
	2 h	–	50.3 ± 4.5	19.2 ± 3.0	29.7 ± 2.4	51.5 ± 5.0[a]	1.2 ± 0.1
		+	41.7 ± 7.2	13.4 ± 3.8	28.3 ± 1.7	46.0 ± 4.3[b]	1.2 ± 0.1
3	15 min	–	55.6 ± 6.9	5.5 ± 2.3	47.8 ± 3.3	26.0 ± 3.1	2.0 ± 0.1
		+	56.3 ± 5.0	10.8 ± 2.8	44.2 ± 3.0	34.9 ± 4.9	1.7 ± 0.1
	2 h	–	65.5 ± 3.1	31.6 ± 3.4[a]	36.4 ± 1.5	65.3 ± 2.1[a]	1.2 ± 0.1
		+	52.1 ± 5.6	20.2 ± 4.0[b]	30.5 ± 2.2	45.9 ± 4.7[b]	1.2 ± 0.1

MOT: Total motility; PROG: Progressive motility; VAP: Average path velocity; LIN: Linearity; ALH: Amplitude of the lateral movement of the head.
[a,b]Different superscripts indicate differences $P < 0.05$ for the SuinFort supplementation effect within day and incubation time.
[†] Indicates SuinFort supplement as a main effect with $P < 0.05$.
[‡] Storage day as a main effect with $P < 0.05$.

Table 4
Flow cytometry results after incubating refrigerated-stored boar semen (1, 2 or 3 days) with SuinFort supplement (+) or no SuinFort (-) at 37 °C. Results are mean ± SEM.

Day[†]	Incubation[†]	SuinFort	Viability[‡]	Apoptotic	Damaged acrosomes	Capacitated	Active mitochondria[‡]
1	15 min	–	74.0 ± 3.2	38.1 ± 8.6	42.8 ± 8.0	1.7 ± 0.7	77.8 ± 3.4
		+	59.3 ± 8.6	53.0 ± 1.4	31.6 ± 5.7	0.6 ± 0.3	63.4 ± 6.7
	2 h	–	59.3 ± 11.3	52.6 ± 6.1	50.6 ± 5.3	3.2 ± 1.1	38.2 ± 3.3
		+	25.4 ± 8.5	62.1 ± 2.7	57.7 ± 5.8	3.5 ± 1.5	22.3 ± 6.1
2	15 min	–	71.5 ± 2.0	70.6 ± 4.9	22.7 ± 4.5	0.6 ± 0.5	60.4 ± 1.9
		+	62.0 ± 7.6	70.7 ± 3.0	23.4 ± 2.4	0.5 ± 0.3	53.9 ± 5.1
	2 h	–	65.7 ± 8.9	73.2 ± 2.9	18.5 ± 4.7	0.6 ± 0.1	50.4 ± 5.6
		+	47.4 ± 8.4	60.4 ± 3.9	19.5 ± 3.4	2.5 ± 2.1	55.9 ± 8.2
3	15 min	–	81.9 ± 3.4	43.3 ± 2.7	23.1 ± 4.4	0.2 ± 0.3	80.7 ± 4.6
		+	76.5 ± 1.3	42.4 ± 2.9	21.2 ± 6.6	0.2 ± 0.3	75.7 ± 3.3
	2 h	–	63.7 ± 9.9	48.8 ± 2.3	54.1 ± 4.1	0.0 ± 0.3	42.9 ± 4.7
		+	51.8 ± 7.1	54.9 ± 3.2	51.1 ± 3.9	0.2 ± 0.3	42.6 ± 7.5

Viability: YO-PRO-1$^-$ cells; Apoptotic: YO-PRO-1$^+$ spermatozoa relative to the PI$^-$ population; Damaged acrosomes: PNA$^+$ spermatozoa; Capacitated: M540$^+$ relative to the YO-PRO-1$^-$ population; Active mitochondria: Mitrotracker$^+$/YO-PRO-1$^-$ spermatozoa.
[†] Day and incubation time (as main effects) significant for all parameters, except Capacitated.
[‡] Effect of SuinFort supplement ($P < 0.05$) as a main effect.

viability increased by day 3 when there was evaluation at 15 min after SuinFort supplementation possibly due to changes in the permeability of the sperm plasmalemma to YO-PRO-1 staining to detect apoptotic changes during storage. Therefore, there was no detectable effects of SuinFort treatment effects on apoptotic-associated or capacitation-assoicated changes or acrosomal damage during the present study.

4. Discussion

Results of the present study indicate there was a positive effect of SuinFort on sow fertility and prolificacy whereas, in contrast to results from previous studies (Bertoldo et al., 2012; De Rensis et al., 2017), there was not an effect of season. Seasonal infertility is an economic problem in many pig breeding systems, with a complex etiology likely related to lesser ovarian activity and lesser oocyte developmental competence when there is heat stress (Bertoldo et al., 2012; De Rensis et al., 2017). There have been previous studies of the seasonal effect the when there was supplementation of oxytocin to the seminal dose and utilization of the semen when there were greater ambient temperatures and there was less fertility when these samples were used as compared with use of non-oxytocin supplemented semen samples (Peña et al., 1998). Duziński et al. (2014) reported there were greater farrowing rates during July through December in Poland when there was supplementation of semen diluents with oxytocin and there was also larger litter sizes, but only during the second half of the year as a result of both oxytocin and PGF2α seminal supplementations. In the present study, the lack of significant seasonal variations and interactions in response to SuinFort-supplementation of semen diluents was likely due to precise control of photoperiod and environmental temperatures in the farrowing facilities which resulted in a lack of fluctuation of nutrient intakes during lactation and the associated decrease of fertility.

The mechanism involved in the previously described effects of oxytocin or PGF2α presumably involves stimulation of uterine contractions, thus, promoting sperm transport to the uterotubal junction (Langendijk et al., 2003, 2005; De Rensis et al., 2012; Okazaki et al., 2014). As a result of oxytocin supplementation of semen diluents, there is a larger sperm reservoir in the oviduct that may have a positive effect on fertility. Variable responses to supplemental oxytocin may result from differences in myometrial response in terms of the direction of contractions, with a potentially increased sperm backflow rather than an enhanced sperm transport to the uterotubal junction depending on the direction of contractility (Langendijk et al., 2003). Effects of supplementation with oxytocin on ovulation cannot be discounted because oxytocin induces endogenous PGF2α release and pre-ovulatory intra-follicular PGF2α concentrations increase rapidly and are maximal at the time of ovulation (Hunter and Poyser, 1985), indicating there is a function for PGF2α in the ovulatory process.

In swine, administration of the GnRH agonists buserelin (Martinat-Botté et al., 2010) or peforelin (Vangroenweghe et al., 2016) by injection, or triptorelin as an intravaginal gel (Knox et al., 2018) resulted in a greater synchronization of time of ovulation among sows as compared with untreated controls. Lecirelin is a super-analog of GnRH which has been used for reducing the length of behavioral estrus and the interval between estrous onset and ovulation in sows (Fries et al., 2010). There are no other reports, however, on the effects of lecirelin or other GnRH analogs when included in boar semen used for insemination.

SuinFort effects can be explained first by the relatively small oxytocin content, which presumably reduced retrograde flow of sperm while promoting efficient contractions helping to establish an optimal oviduct sperm reservoir. Furthermore, the supplementation with lecirelin likely induced a greater synchronization in ovulation among sows as compared with what occurred with AI with the control sample. A greater sperm transport to the oviduct and improved timing of insemination relative to the time of ovulation would be expected to result in enhanced fertility. Additionally, SuinFort contains caffeine, which has been used frequently as a seminal supplement to improve sperm motility in fresh and cryopreserved semen samples (Miyamoto and Nishikawa, 1980; Funahashi and Nagai, 2001; Bury et al., 2017), and possibly exerts immunomodulatory effects in the uterus. Supplementation of semen diluents with caffeine apparently reduces sperm phagocytosis by uterine neutrophils and the uterine inflammatory response to AI (Yamaguchi et al., 2009, 2013), improving the fertility when there is use of frozen-thawed semen for AI.

In addition to effects on the uterine milieu (Matthijs et al., 2003; Yamaguchi et al., 2013) caffeine causes the inhibition of enzymes including nucleotide phosphodiesterase, resulting in increased intracellular cAMP concentrations which are essential for the maintenance of sperm motility (Tash and Means, 1983). Caffeine also induces a capacitation-associated reaction and spontaneous acrosomal reaction in boar spermatozoa as indicated by results from use of the CTC assay (Funahashi and Nagai, 2001). In the present *in vitro* experiment, the addition of SuinFort to the semen diluents did not result in an enhanced motility nor induce an increase in the proportion of capacitated or acrosome-reacted spermatozoa. This outcome likely was a consequence of the use of liquid semen rather than frozen-thawed semen in the present study for AI. Interestingly, sperm linearity, which was less at the beginning of the incubation (possibly an effect of warming the sample from 15 to 37 °C), and recovered more rapidly with the supplementation of semen diluents with SuinFort, however, there was a subsequent decrease (at 2 h) in linearity values in the SuinFort-supplemented samples which was greater than those for the control samples. It is possible that the caffeine supplementation in the present study resulted in this negative effect on linearity in SuinFort-supplemented samples because in other studies there were no adverse effects of oxytocin (Okazaki et al., 2014) or GnRH analog treatments (Mizera et al., 2018) on spermatozoa *in vitro*. These *in vitro* effects with SuinFort supplementation of semen diluent on sperm were small and may not be important when fertility is evaluated *in vivo*. A direct stimulatory effect of SuinFort on boar spermatozoa cannot be discounted. The most likely explanation of the observed effects of SuinFort, therefore, is a direct effect of this semen supplement on the uterus possibly as a result of enhanced myometrial contractility.

In conclusion, results of the present study indicate the seminal supplementation with SuinFort had a positive effect on sow fertility when there was use of artificial insemination. Supplementation of seminal doses with a combination of oxytocin, lecirelin, and caffeine in the SuinFort semen supplement resulted in a consistent effect on sow fertility throughout the year. Furthermore, because SuinFort is added to the semen, it avoids additional management of the sow as would occur if the constituents of the SuinFort supplement were administered to sows before AI.

This study involves a patent (ES2608935) filed by J. C. Domínguez and F. Martínez-Pastor. This study has no other conflict of interests.

Declaration of Competing Interest

This study involves a patent (ES2608935) filed by J. C. Domínguez and F. Martínez-Pastor. This study has no other conflict of interests.

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