Fixed time artificial insemination in gilts and sows. Tools, schedules and efficacy

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Fixed time insemination is a strategy that may facilitate batch management of swine units. After briefly discussing the advantages and disadvantages of the drugs available to induce ovulation in swine and their optimal timing of administration, the reproductive performance of a scheme combining ovulation induction and a single fixed time artificial insemination will be described for gilts and sows. In gilts, this single fixed time insemination regimen includes a GnRH agonist (buserelin) injection at 115-120h after the last feeding of altrenogest, followed 30-33h later by the single insemination. In sows, buserelin is injected 83-89h after weaning and the single fixed time insemination is again performed 30-33h later. Reproductive performance of this scheme was evaluated in two field trials, involving different genetic lines maintained on several farms in several countries, and using females from the same farms bred twice at detected estrus as negative controls. In gilts as well as in multiparous sows, fertility and prolificacy (total born as well as live born) were similar following a single fixed time insemination compared to the negative controls. In these two animal types, this breeding schedule was robust as none of the zootechnical or management factors analyzed interacted with its efficacy. As the number of primiparous sows included in this field trial was limited, further studies will be warranted to establish whether this breeding schedule may be also used in such females (particularly those with short lactations).

Overall, this single fixed time breeding system may be useful for farmers due to reduction in labor and semen costs. In addition, in the longer term, it may facilitate the implementation of techniques that maximize the diffusion of genetic merit from elite boars through use of frozen or sexed semen that need a close synchrony between insemination and ovulation.

Introduction

A pre-requisite to obtain high fertility and prolificacy in swine is to perform insemination with fresh semen less than 24h before ovulation (Waberski *et al*. 1994, Nissen *et al*. 1997, Steverinck *et al*. 1997). As reproductive management of pig farms increasingly relies on batch production systems, altrenogest administration to gilts and weaning of sows are widely used to synchronize

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estrus. However, owing to the variability in the duration of the follicular phase until ovulation, labor intensive estrus detection and multiple inseminations are commonly used to ensure high fertility. An alternative strategy would be to induce ovulation and then time the artificial insemination (AI) relative to ovulation to make sure that the insemination to ovulation interval does not exceed 24h. The benefits of such a strategy would be a fully integrated breeding schedule with the following characteristics: no time and labor spent in estrus detection and reduced semen costs and labor associated with the insemination process, itself. The aim of this review is to summarize our understanding of the potential and limits of fixed time AI in swine, and describe the reproductive performance generated by a breeding strategy involving ovulation induction combined with a single fixed time AI in gilts as well as in sows.

Which compounds and routes may be used for inducing ovulation?

Table 1 contains a summary of different molecules that have the ability to induce ovulation and have been used in fixed time insemination programs

Human chorionic gonadotropin (hCG) is a highly glycosylated placental human protein that acts as an LH agonist in all farm animal species. Owing to the extensive glycosylation carried on its CTP tail, hCG displays an extended half-life reaching several days as opposed to a few hours for native LH. The minimum effective dose of hCG is 500 IU (Ziecik *et al*. 1987). The interval between hCG administration and ovulation ranges between 36 and 40h (Dziuk & Baker 1962).

Porcine pituitary LH (pLH) is purified and marketed in some countries by Bioniche Animal Health. The recommended clinical dose is 5mg (Bennett-Stewart *et al*. 2007). This dose generates an LH peak of similar duration, but of higher amplitude, than the natural LH surge (Degenstein *et al*. 2008), and was shown to successfully induce and synchronize ovulation of weaned sows, 36 to 44h after pLH injection (Cassar *et al*. 2005).

Parenteral administration of gonadotropin releasing hormone (GnRH) analogues such as buserelin (MSD Animal Health), goserelin and depherelin (Veyx Pharma) triggers ovulatory LH

Table 1: Characteristics of the different ovulation inducing drugs discussed in this review

	hCG	pLH	Parenteral GnRH agonist	Intra-vaginal GnRH agonist
Dose used	500 IU	5 mg	10 μg buserelin, 20 μg goserelin, 50 μg depherelin	100-200 μg triptorelin
Pros	- Efficient ovulation induction - Long half life	- Efficient ovulation induction	- Pure synthetic peptide - Efficient ovulation induction - Buserelin widely registered for this indication in gilts (except US/Canada) and registration in sows on going	- Pure synthetic peptide - Novel administration route - Efficient ovulation induction
Cons	- Extracted molecule - Registered only in cattle in most countries	- Extracted molecule - Not registered in most countries		- Only available in the US - High amounts of agonist needed

surges that generally induce and synchronize ovulation 35 to 44 hours later (Van Kaufmann& Holtz 1982, Brüssow *et al.* 2009). Dose finding studies conducted in gilts with goserelin (Brüssow *et al.* 2007) and buserelin (Van Kaufmann & Holtz 1982) and in sows with buserelin (Driancourt *et al.* 2013) showed that time to ovulation as well as features of the induced LH surge were unrelated to the dose of GnRH injected, once a threshold dose had been reached (Van Kaufmann & Holtz 1982; Driancourt *et al.* 2013).

Intra vaginal administration of a methyl cellulose (1.2 to 1.5%) based gel containing 100 μg of triptorelin (Ovugel® from JBS United) induces an LH surge 4 to 12h post treatment and synchronizes ovulation 36 to 48 hours later (Stewart *et al.* 2010). However, when tested in larger number of sows, the ovulation induction rate of 58% over a 48h time period with such a gel was limited (Knox *et al.* 2011). Increasing the amount of triptorelin to 200 μg was required to generate a tighter synchronization of ovulation (Knox *et al.* 2011).

Lamprey GnRH-III is a decapeptide that, in barrows, raises LH concentrations when high doses are used (10 μg/kg) (Barretero-Herandez *et al.* 2010). In contrast, in gilts, LH concentrations are unaffected by injection of 150μg of lamprey GnRH-III (Brüssow *et al.* 2010). Hence, Lamprey GnRH-III cannot be considered as an ovulation inducer.

Is the dose of ovulation inducer critical?

Two end points, namely i) the time interval between treatment and ovulation, and ii) fertility following treatment, have been used to address this question. Most data were generated following buserelin injection to gilts and multiparous sows. Since ovulation starts as soon as LH concentrations exceed a threshold value, all trials assessing the optimal dose of ovulation inducer demonstrated a biphasic response pattern. As doses were progressively increased, an increasing proportion of females ovulated, until a plateau was reached. Such a pattern was very obvious in the comprehensive dose finding study conducted with buserelin in gilts (Van Kaufmann& Holtz 1982). This conclusion was confirmed when the effects of three doses of buserelin, 6, 10 or 16 μg injected at 77 hours after weaning, were compared in sows (Driancourt *et al.* 2013). In multiparous sows, 100% of ovulation induction was obtained irrespective of the dose administered. Neither the features of the LH surge, nor the ovulation rate, nor the features of early embryonic development were affected by the dose of buserelin. However 3 of 15 sows treated with 6 μg buserelin had 3 or more cystic follicles, while no ovarian cysts were observed in the other treatments. Sows in which multiple cysts were detected displayed a blunted LH surge. Interestingly, in primiparous sows, a different pattern of response was observed. The percentage of sows responding to ovulation induction was consistently reduced with only 50, 50 and 67% responding to treatment with 6, 10 and 16 μg buserelin respectively (Driancourt *et al.* 2013). The limited ovulation induction rates of these females may have been caused by treatment at 77h after weaning, a time that may be too early for primiparous sows. The lack of dose response observed in primiparous sows shows that increasing the dose of ovulation inducer cannot correct an improper time of administration.

Are all compounds equally effective?

This is an open question as a systematic comparison of the efficacy of all families of ovulation inducers using end points such as ability to induce ovulation, tightness of ovulation, fertility and prolificacy has never been conducted. A compilation of the reports describing the time of ovulation after injection shows that all compounds induce ovulation within the same time frame. The only possible exception is the vaginal gel formulation containing triptorelin that needs 4 to 12 hours to trigger the ovulatory LH surge (Stewart *et al.* 2010).

When the potency of the different GnRH agonists tested was compared using the recommended clinical dose, differences were striking, as only 10 μg of buserelin was needed to trigger a full LH surge compared with 20 μg goserelin and 50 μg depherelin. The very high concentrations of GnRH agonist, 100 to 200 μg triptorelin, needed in the vaginal formulation may be explained by proteolysis occurring in the vaginal cavity and limited bio-availability of peptides via this administration route.

When using fertility and prolificacy as clinical endpoints to compare efficacy of different ovulation inducers, GnRH agonists such as depherelin tend to deliver a slightly improved reproductive performance compared to hCG (Brussow *et al.* 1996, Kauffold *et al.* 2007).

Is the time of ovulation induction critical?

In gilts, ovulation has almost always been induced following an altrenogest-based synchronization treatment of 20 mg for 18 days in Europe. Two treatment schemes have been described (Figure 1). In the first one, 800 – 1000 IU eCG is administrated after the end of altrenogest and ovulation is induced 102-104 hours after the end of altrenogest (Brüssow *et al.* 2009). In the other one, no exogenous gonadotropins are injected and ovulation is induced with buserelin between 104 and 120 hours after the end of altrenogest (Martinat-Botte *et al.* 2010). Both schemes used two fixed time inseminations.

In weaned sows, four treatment schedules have been devised (Figure 2). The first scheme involves injection of 600 IU eCG at weaning combined with ovulation induction with 5 mg pLH 80 hours later (Cassar *et al.* 2005). The second scheme also includes an injection of eCG, 24 hours after weaning followed by ovulation induction 56 to 80 hours later by 50 μg depherelin (Brüssow *et al.* 2009). The last two treatment schemes (Figure 2) rely on natural resumption of follicular growth after weaning, and induction of ovulation (with 10 μg buserelin or 100

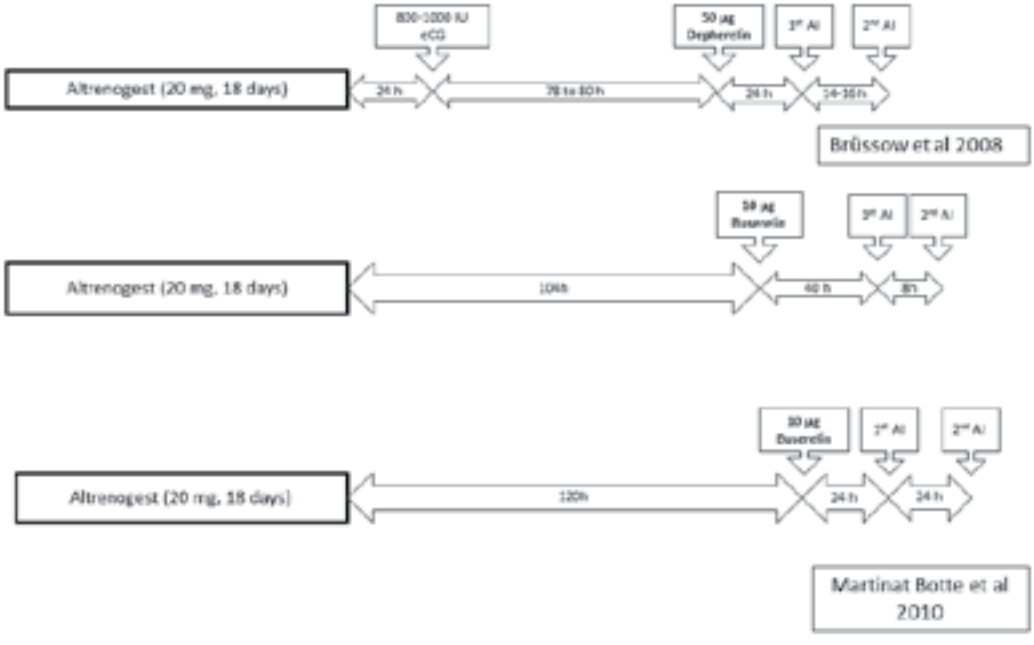

Fig. 1 Summary figures describing the different fixed time insemination breeding strategies previously evaluated in gilts (Brüssow *et al.* 2008, Martinat Botte *et al.* 2010)

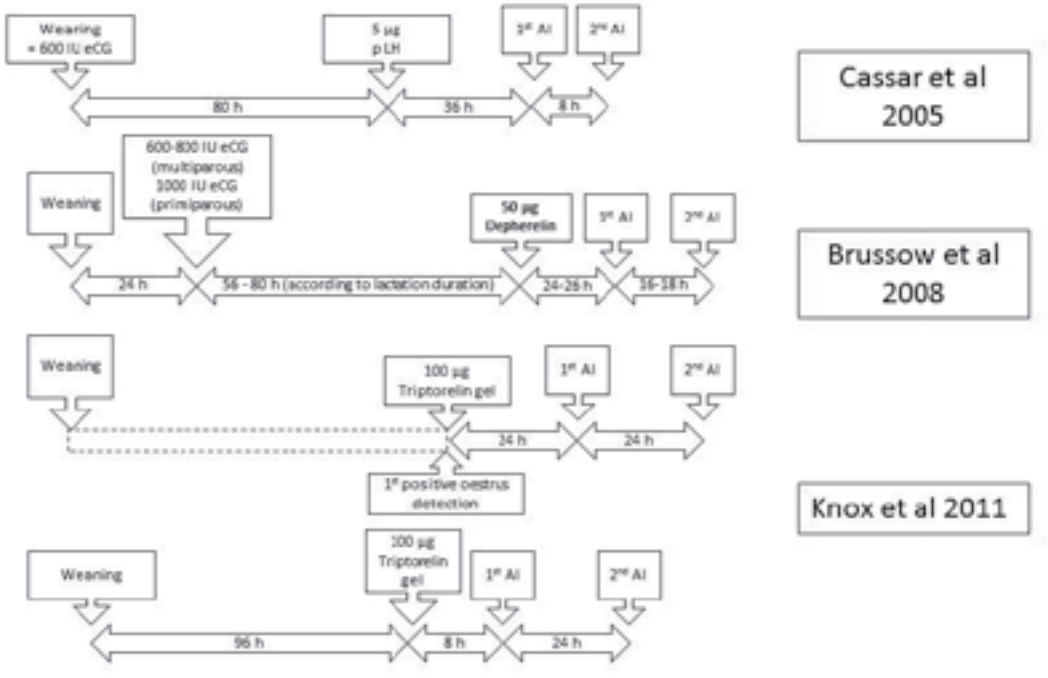

Fig. 2 Summary figure describing the different fixed time insemination breeding strategies previously evaluated in sows (Cassar *et al*. 2005, Brüssow *et al*. 2008, Knox *et al*. 2011)

µg triptotrelin) at 94 or 104 hours after weaning (Martinat-Botte *et al*. 2010) or 96 hours after weaning or at the first positive estrus detection (Knox *et al*. 2011), respectively, followed by two inseminations. Comparisons of reproductive performance of these treatment schedules demonstrated the following.

In gilts, inducing ovulation 104 to 120 hours after the end of altrenogest treatment delivers optimal fertility and prolificacy and adding exogenous gonadotropins in the scheme is not useful.

In weaned untreated multiparous sows, reproductive performance is better when ovulation induction is achieved around 94-96 hours post weaning than later on.

In weaned sows treated with eCG, the ideal time for ovulation induction is 80 hours post weaning.

What are the risks of ovulation induction?

Ovulation induction may fail for two main reasons. Firstly, the ovulatory signal may not reach the threshold value needed to activate the ovulatory cascade, or, secondly, it may not be given at the right time.

Induction of a sub-optimal LH surge: This is obviously not a risk when exogenous LH is used for ovulation induction, as the LH profile induced by pLH administration is quite similar to the endogenous LH surge (Degenstein *et al*. 2008). If the recommended clinical dose of GnRH agonist is used, a sub-optimal LH surge does not commonly occur in gilts and multiparous sows. In contrast, in primiparous females that do not display a fully functional pituitary response to GnRH owing to their negative energy balance, GnRH agonists may trigger an attenuated surge. This may result in anovulation of part of the ovulatory cohort or in cystic follicles that may detrimentally affect fertility (Castagna *et al*. 2004)

Induction of a premature LH surge: If triggered too early during the follicular phase, when some follicles have not acquired their full sensitivity to LH, an LH surge may fail to trigger ovulation or induce ovulation of immature follicles. Indeed, following treatment with a triptorelin gel, only 63% of sows with follicles smaller than 6.5 mm in diameter ovulated as opposed to 96% of those with larger follicles (Knox *et al*. 2011). In another study (Driancourt, unpublished observations), when premature ovulation induction of gilts was triggered by injecting 10 μg buserelin 96 hours following the end of altrenogest treatment, the proportion of females ovulating was reduced below 75 % and the proportion of females displaying at least one cystic follicle was increased.

Late administration of the ovulation inducing drug: This may occur if the ovulation inducing compound is injected after initiation of the endogenous LH surge. While this is unlikely to have detrimental effects on the ovulation process, per se, fixed time AI of such females will be done during late estrus, and post ovulatory inseminations are associated with reduced farrowing rates (Rozeboom *et al*. 1997) .

Which strategies provide good reproductive performance following a single fixed time AI?

Novel treatment schedules that combine ovulation induction with buserelin followed by a single fixed-time AI in gilts and sows are presented in Figure 3. All previously published treatment schedules except a few abstracts and one group in the study by Cassar *et al*. (2005) have used two fixed time AIs following ovulation induction. Use of a breeding system only implementing one AI carries the risk of reducing fertility by 12% and prolificacy by 1.4 piglets (Flowers & Alhusen 1992). As shown below, this assumption is not valid when suitable timings of ovulation induction and insemination are applied.

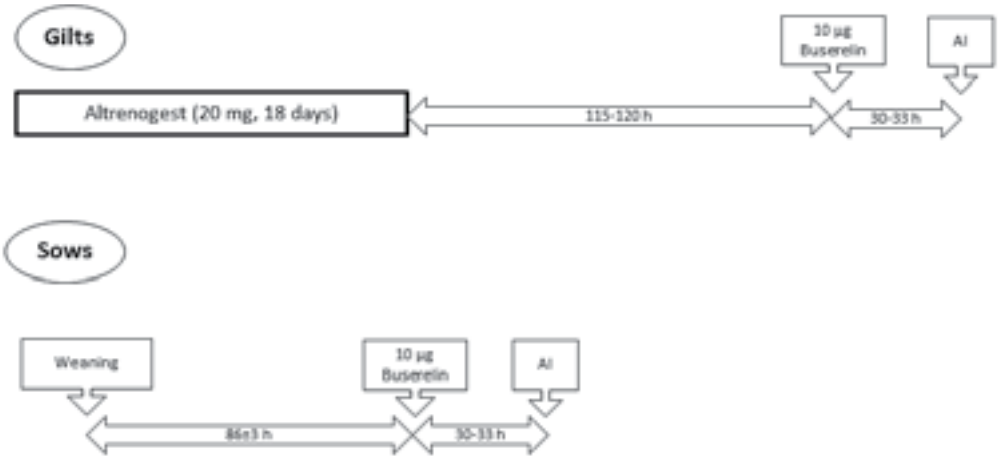

Fig. 3 Summary figure describing the two schedules described in details in this review using a single fixed time insemination strategy following buserelin administration to gilts (top panel) or sows (bottom panel)

What technical performance can be obtained in gilts with a treatment scheme involving a single fixed time AI?

This was investigated in a large clinical trial conducted on 3 farms in Spain and on 5 farms in France and including gilts of different genetic backgrounds. Full details on the zootechnical and management features of each of these farms are presented in Table 2. Pubertal gilts (n = 436)

Table 2. Zootechnical and management data of the farms involved in the single fixed time insemination trial in gilts.

Farm number	Farm location	Farm type	Farm size (breeding females)	Farm genetics (females)	Previous average farm farrowing rate	Previous average farm prolificacy (live born)	Recurrence of breeding batches
1	France	Farrow to finish	364	Naima	79%	12	3 weeks
2	France	Farrow to finish	200	Naima	78%	13.2	5 weeks
3	France	Farrow to finish	240	Youna	87%	12.8	5 weeks
4	France	Farrow to finish	630	Dalland	92%	12.8	3 weeks
5	France	Farrow to finish	294	Large White (LW)	91%	13.8	3 weeks
6	Spain	Farrow	714	LW X Landrace	80%	12-14	weekly
7	Spain	Farrow	630	Landrace X LW X Duroc	83%	12-13	weekly
8	Spain	Farrow	490	PIC	82%	12	3 weeks

that had their estrous cycles synchronized by daily feeding of 20 mg of altrenogest for 18 days were randomly allocated to a control group (n = 218) or to a treated group (n = 218). Control gilts were bred by two A.I. 12 hours apart at detected estrus after altrenogest while buserelin-treated gilts received 10 μg buserelin 115-120 hours after altrenogest and a single fixed time AI 30-33 hours later. Fifty three gilts were excluded owing to inconsistent intake of altrenogest in group fed animals. Farrowing and prolificacy data are therefore based on 199 controls and 184 treated gilts. As shown in Table 3, farrowing rate and prolificacy were similar in both groups, with no statistical differences between groups.

To check whether this breeding schedule was robust, the significance of interactions between body condition score, number of cycles prior to inclusion and treatment was tested. None of these factors was shown to significantly interact with fertility and prolificacy. Finally, to assess the flexibility of the treatment schedule, interactions between fertility, prolificacy and treatment features such as the time interval between end of altrenogest feeding and buserelin injection, 115-120 hours, and the time interval between buserelin and insemination, 30-33 hours, were tested. Although these results need to be taken cautiously owing to limited numbers in each class, fertility was numerically higher when the end of altrenogest to buserelin interval was 119 to 120h (76/91: 84%) than when it was 117 to 118 hours (29/41: 71%). Prolificacy was also

Table 3. Reproductive performance observed in a clinical trial in gilts, comparing fertility and prolificacy following either buserelin (injected at 115-120 h post altrenogest) and a single fixed time insemination (30-33 h after buserelin) or two inseminations (12 h apart) at detected estrus ("controls")

	Buserelin treated	Control
Nb of gilts	184	199
Farrowing rate (%)	78.8%	80.9%
Total piglets born (sem)	13.1 ± 0.3	12.9 ± 0.3
Piglets born alive	12.1 ± 0.3	11.9 ± 0.3

improved when gilts were inseminated later, as shown by the significant ($P < 0.01$) regression lines linking the number of live born (Y) and total born (Z) piglets and time ($Y = 1.41t-156$; $Z = 1.54t-170$). In contrast, within buserelin-treated gilts, fertility and prolificacy were not significantly altered when end of altrenogest to buserelin injection intervals were compared.

What technical performance may be obtained in sows with a treatment scheme involving a single fixed time insemination?

This question was addressed in a large clinical trial conducted on two farms each in Spain, Germany and France involving sows of different genetic backgrounds, parities and durations of lactation. Zootechnical and management data of all 6 farms are presented in Table 4. This trial compared reproductive performance of weaned sows ($N = 419$) given 10 ug buserelin at 86 ± 3 hours after weaning and bred with a single fixed time insemination 33 hours later (174 primiparous and 39 primiparous) with a traditional insemination regimen of 2 A.I. matings administered 12 ± 4 hours apart relative to detected estrus (168 multiparous and 38 primiparous). Within each farm, all sows of the selected batches were eligible for inclusion except those that lost over 30% fat during lactation. This led to the exclusion of 14% and 5.5% of the primiparous and multiparous sows, respectively.

Farrowing rate, total number born, and number born alive were the key end points. Possible interactions between treatment and duration of lactation or fat loss during lactation were also assessed.

All control sows except 6 (200/206; 97.1%) displayed estrus and were inseminated twice between days 4 and 7 post weaning. Because buserelin-treated females were only bred if they were in estrus when insemination was scheduled, the insemination rate of buserelin-treated females only reached 192/213 (90.1%; $P < 0.01$), with a minimal insemination rate (82%) recorded in primiparous sows (Table 5). Interestingly, when the effects of lactation duration on insemination rate were analyzed, a trend towards a duration of lactation by treatment interaction ($P = 0.09$) was demonstrated as, buserelin-treated sows had reduced insemination rates (83.7%) compared to control sows (98.8%) when weaned after a lactation shorter than 24 days.

Table 4. Zootechnical and management features of the farms involved in the single fixed time insemination trial in sows

Farm location	Farm type	Farm size (females bred)	Farm genetics (females)	Lactation duration target (days)	Previous average farm farrowing rate	Previous average farm prolificacy (live born)	Recurrence of breeding batches	Mean farm parity
France	Farrow to finish	582	Naima	21	94.3%	13.2	weekly	3.68
France	Farrow to finish	310	Dalland	21	92.4%	13.2	3 weeks	4.16
Spain	Farrow to finish	800	Crossbred	28	81.3%	11.9	3 weeks	3.69
Spain	Farrow to finish	370	Crossbred	28	81.1%	11.5	3 weeks	3.02
Germany	Farrow to finish	291	PIC	21	84.0%	11.6	weekly	3.43
Germany	Farrow to finish	400	ADN	28	92.0%	11.4	3 weeks	3.55

Table 5. Reproductive performance observed in a clinical trial in weaned sows, comparing fertility and prolificacy following either buserelin (injected at 86 ± 3 h after weaning) and a single fixed time insemination (30-33 h after buserelin) or two inseminations (12 h apart) at detected estrus ("controls")

	Control		Treated	
	Multiparous	Primiparous	Multiparous	Primiparous
Number of sows	168	38	174	39
Insemination rate	97.6%	94.7%	91.9%*	82.0%
	(164/168)	(36/38)	(160/174)	(32/39)
Farrowing rate	84.1%	86.1%	88.1%	78.1%
	(138/164)	(31/36)	(141/160)	(25/32)
Total number of piglets born	13.89 ± 0.26	12.83 ± 0.61	13.69 ± 0.31	13.16 ± 0.80
Number of live born piglets	12.85 ± 0.25	12.29 ± 0.56	12.46 ± 0.27	12.60 ± 0.76

*P = 0.02 vs control multiparous

Full details on the effects of treatment and parity on reproductive performance are presented in Table 5. Fertility was similar in control (169/200; 84.5%) and treated sows (166/192; 87%). This conclusion was not affected by parity (multiparous sows: control 84.1% vs buserelin-treated 88.1%; and primiparous sows: control 86.1% vs buserelin-treated 78.1%). In multiparous sows, analysis of the main factors possibly interacting with treatment to modulate fertility failed to identify significant interactions between treatment and parity, lactation length or fat loss during lactation. Noteworthy was the observation that fertility of primiparous sows with lactation lengths less than 24 days was low in both treatment groups (controls: 75% vs treated 44%) with no significant difference between these ratios owing to small numbers.

The total numbers of piglets born as well as those born alive were also unaffected by treatment (Table 5). This conclusion was also supported when performance of multiparous and primiparous sows were considered separately (Table 5). In addition, significant interactions between treatment and duration of lactation or fat loss during lactation were never demonstrated for prolificacy.

Conclusions and need for further investigations...

Breeding gilts and sows, with a single fixed-time artificial insemination (FT-AI) 30-33 hours after injection of 10 μg buserelin delivers consistent reproductive performance. This treatment schedule has three main advantages. Firstly, as the insemination is done at a fixed time, estrus detection is not required, therefore saving time and labor. Flowers & Alhusen (1992) estimated that 10 minutes are required per female for detecting estrus at each breeding cycle. In addition minimizing estrus detection will reduce risks of accidents to farm personnel when handling boars. Secondly, the single-FT-AI treatment schedule will generate savings on semen costs, one semen dose used compared with two or more. These savings will counterbalance the treatment costs. Finally, as only one insemination is performed, the time spent inseminating will be reduced by at least half. Flowers & Alhusen (1992) calculated that 3.4 to 10 minutes were needed for each insemination. The good performance of this timed breeding system confirms and extends earlier claims suggesting that acceptable fertility and prolificacy could be obtained with schemes using two fixed time inseminations after ovulation induction with

a range of ovulation inducing compounds such as hCG, pLH and other GnRH agonists or following ovulation induction at initiation of estrus.

When considering the results of the two clinical trials collectively, the main conclusions are as follows: (1) the breeding schedules for both gilts and multiparous sows using buserelin and a single fixed-time insemination deliver results that in most cases are similar to those obtained with conventional AI regimens based on two inseminations after detected estrus; (2) these schedules need to be followed closely as small shifts in the time of buserelin administration may alter fertility and prolificacy; and (3) responses of early weaned and primiparous sows to this timed insemination schedule need additional research to be optimized fully. Firstly, early weaned females, irrespective of their parity, displayed reduced insemination rates because some of them were not displaying estrus at the time due for insemination. In addition, reduced farrowing rates were also detected in early weaned primiparous sows. Consequently, in systems using short lactations, such as in the US, the timing of ovulation induction may need to be delayed to allow the pituitary to regain full responsiveness to GnRH and to allow follicles to reach a suitable size and maturation. Secondly, sows losing more than 30% dorsal fat during lactation were excluded from the clinical trial. The performance of the present breeding schedule could not therefore be evaluated in such females. In both sub populations, it is likely that the severe negative energy balance experienced during lactation (Zak *et al*. 1997, Quesnel *et al*. 1998, Hoving *et al*. 2012) may explain why a different treatment scheme should be devised. Treatment options for such females could (1) delay buserelin injection by one day, therefore allowing more time for recovery of full pituitary responsiveness and more time for follicular growth and maturation, (2) wait until initiation of estrus to inject buserelin or (3) inject eCG or PG 600 at weaning to support follicular growth throughout the follicular phase. The relative merits of each approach will need to be assessed in further studies.

The single FT-AI breeding schedule will also need to be also evaluated in more sophisticated breeding systems aiming to optimally spread genetic merit by using semen from elite boars. Confirmation of the good reproductive performance of sows submitted to deep insemination using reduced sperm numbers following the fixed time insemination schedule described above may be a useful first step in that direction. In the longer term, the very predictable ovulation induction obtained following injection of buserelin might be valuable to minimize the insemination to ovulation interval, hence allowing use of frozen or sexed semen. Further research will be needed to document whether this is possible.

Declaration of interest: MAD is a permanent employee of MSD Animal Health Innovation

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