Studies on fixed-time ovulation induction in the pig

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A technology that allows for manipulating of oestrus and ovulation, and would then also allow for fixed-time insemination, can be of great benefit for swine farms that operate using sow batch management due, at least in part, to savings in labour and the production of large batches of evenly developed pigs. Thanks to the current knowledge on endocrine regulation of follicle development and ovulation, and the availability of numerous reproductively active substances such a technology is now available. It covers procedures for synchronising oestrus based on the use of altrenogest in gilts and of batch-wise weaning in sows, for stimulating follicle development using eCG and for inducing of ovulation using hCG or LH as well as GnRH analogues. While the procedures for oestrus synchronisation stand alone, other procedures require additional treatments. If fixed-time insemination is the goal, oestrus needs to be synchronised and follicular development and ovulation induced by the use of GnRH analogues and hCG with ovulation occurring within 36-42 hrs. It is a general recommendation to inseminate those animals twice, i.e. 24 and 40 hrs after ovulation induction. However, the aforementioned technology requires healthy animals and a solid management and cannot be used to compensate for poor management.

Introduction

More than half a century ago research efforts were made to synchronise the oestrous cycle and ovulation in pigs with the ultimate goal of fixed-time artificial insemination (AI). Tanabe *et al.* (1949) were the first to treat pigs with equine chorionic gonadotrophin (eCG) and sheep pituitary extracts in order to stimulate follicle development and ovulation. Further studies using methallibure to suppress follicular growth followed by treatment with eCG to stimulate follicle development and human chorionic gonadotrophin (hCG) for inducing ovulation (Polge & Day 1969), and those conducted by Hunter (1967,1974) on ovulation induction as well as on follicle and oocyte development were milestones in the development of procedures to manipulate the oestrus cycle in pigs. Results of these early studies facilitated Eastern-European, particularly East-German research efforts into those procedures starting in 1970 at a time when the farm inventories were growing and there was an increasing need for this kind of biotechnology for management purposes. The general approach was to manipulate female reproductive functions, such as follicular development as well as ovulation and parturition. However, the goal was to

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mimic what occurs physiologically in the female pig. The ultimate goal was to synchronise all reproductive processes with the advantage of periodic and batch-wise AI, parturition as well as weaning, thereby enabling the practise of all-in-all-out and to produce large groups of pig in the same reproductive state with the same health and immunisation status. Indicative of the success made over the years in reproductive biotechnology in 1990, controlled reproduction was used on 86 % of the 1.1 million breeding sows and gilts in East-Germany. It has been learned that research into and the practical use of biotechnological procedures requires understanding of reproductive processes and the availability of appropriate substances (i.e. hormones or hormonally active substances). While those substances are available in general to manipulate almost all key reproductive processes in the female pig, they are not equally available since their use in practice requires national approval. As compared to Europe, hormones or hormonally active substances are still rarely used in the North and South American as well as the Asian swine industry. However, during the last decade due to increased costs for labour, feed and energy resulted in new interest in using those substances that allow for manipulating reproductive processes in the female pig.

Follicle development and ovulation

Follicular development in gilts and sows has been reviewed in detail elsewhere (Prunier & Quesnel 2000, Schwartz *et al.* 2008). In gilts the follicle cohort destined to ovulate is stimulated by increased post-ovulatory FSH, but under the influence of the high luteal progesterone (P4) concentration during dioestrus follicles do not grow to ovulatory size. Only after luteolysis when P4 is low and thus does not negatively feedback on gonadotrophin synthesis, follicles finally grow from 4 mm to ovulatory size within 4-6 days. However, there is a well-defined balance between stimulatory (e.g. LH and FSH) and inhibitory (e.g. P4 and inhibin) factors that favours pre-ovulatory follicle development. As has been shown after gonadotrophin deprivation, FSH is necessary to support follicle development beyond 2-3 mm and LH beyond 4 mm (Driancourt *et al.* 1995). Once the follicles reach a larger preovulatory stage FSH declines (Guthrie & Bolt 1990) and LH pulse secretion changes from luteal (high amplitude - low frequency) to follicular patterns (low amplitude - high frequency). Final stage of follicular development is associated with decreased FSH and increased LH receptor expression along with increased production of oestradiol, as well as increased fluid accumulation within the follicular cavity. Increased pre-ovulatory oestradiol finally elicits the GnRH-mediated LH release from the pituitary, thereby initiating ovulation and the release of a mature oocyte.

Sows commonly have a lactational anoestrus. During lactation and before weaning, follicles exhibit a wave-like pattern of growth, and emerge from a cohort of 20-30 follicles of 2 mm that grow to not larger than 5 mm (Lucy *et al.* 2001). This is because suckling inhibits secretion of GnRH and subsequently LH due to a concerted action of prolactin, oxytocin and endogenous opioid peptides that prevent final growth of these follicles to reach ovulatory size before weaning (Varley & Foxcroft 1990). Once the piglets are weaned, follicles start to grow to 7-8 mm before ovulation. Initially FSH increases and then decreases at weaning. Basal LH and LH pulse frequency increases; these are key regulators of post-weaning follicle development which affect the weaning to oestrus interval (van de Brand *et al.* 2000). Furthermore, adrenal hormones and nutritional mediators such as glucose, insulin and free fatty acids, as well as endogenous opioids and leptin have been shown to have an influence on post-weaning follicle development and ovulation. In conclusion, follicle development including the final ovulatory follicle growth and maturation, as well as ovulation itself, requires fine-tuned hormonal events. Understanding these mechanisms is necessary in any attempt to manipulate the reproductive process in female swine.

Manipulation of follicle development and ovulation

Synchronisation of oestrus

In a batch farrowing system, oestrus in sows is naturally synchronised by weaning. Oestrous cycles of gilts need to be synchronised when they are introduced into such systems. For this purpose, protocols based on either suppression of follicle development and/or mimicking the luteal phase are available (reviewed by Estill 2000). Progesterone and its synthetic derivates did not prove fully effective for oestrus synchronisation. In contrast, non-steroidal substances such as methallibure (Aimax, ICI 33828, Suisynchron®) have been shown to yield good results, but were banned by US and European countries before being commercially available for the swine industry because of their teratogenic effects. In East-Germany, zinc-methallibure (Siusynchron®) was successfully used in hundreds of thousands of gilts between 1973 and 1989, but was then also banned and replaced by allyltrenbolone (Hühn *et al.* 1996). At present, allyltrenbolone (altrenogest, as Regumate® in Europe and Matrix® in North America) is the only licensed substance with progestagene type effects to be used in female pigs in Europe and in North America. If it is given orally and fed in a dose of 15 to 20 mg/day/gilt over a period of 14 to 18 days, it has been proven effective at suppressing follicle development. Recommendations on the daily dose of altrenogest and the duration of administration differ between countries. Production sites in France and also some in Germany prefer feeding 20 mg/day/gilt for 18 days (Martinat-Botte *et al.* 1990). Other sites in Germany use feeding altrenogest in doses of 16-20 mg/day/gilt over 15 days and achieved good fertility results (Hühn *et al.* 1996). For North America, the general recommendation is 15 mg/day/gilt for 14 days. Feeding of altrenogest for a shorter period is possible, if the stage of oestrous cycle is known at first administration (Kirkwood 1999). Gilts usually show oestrus within 5 to 7 days after altrenogest withdrawal (Martinat-Botte *et al.* 1990, Hühn *et al.* 1996). Other approaches have been to include progesterone or GnRH agonists in a slow release device during a defined time, in conjunction with hCG or oestrogen to extend the lifespan of the corpora lutea, and the 'breed-and-abort' procedure using $PGF_{2\alpha}$. However, all of the aforementioned procedures except those based on the use of altrenogest are not currently used in the swine industry.

Stimulation of follicular development

Though synchronisation of oestrus is possible, with weaning group of sows or treatment with altrenogest in gilts, the onsets of oestrus can however still spread over a week. This is due, at least in part, to insufficient follicle development. Gonadotrophins may then be used after weaning or after altrenogest in order to stimulate this follicle development and to achieve a better synchronisation effect.

Equine CG, which is a glycoprotein similar to equine pituitary LH, has been proven to have superior effects on follicle development in pigs. This gonadotrophin exhibits both LH- and FSH-like activities (Farmer & Papkoff 1979) with a FSH/LH activity ratio that differs between 0.14 to 0.31 as estimated by bioassays conducted in rats and mice (Bergfeld & Haring 1983). A different ratio of FSH *versus* LH activity may account for variations in the ovarian response seen in pigs after use of different eCG batches (Bergfeld & Haring 1983, Ciller *et al.* 2008), and should be considered when analysing the effectiveness of different treatment protocols that are based on eCG. As evident from numerous East-German experiments, 800 to 1,000 IU of eCG are most effective at stimulating follicle development in gilts and 600 to 1,000 IU eCG in sows. However, within- and between-farm differences occur, as indicated by variations in the ovarian response of sows that received the same eCG treatment but were located at different

farms. This observation needs to be considered when determining an optimal dose of eCG (Bergfeld *et al.* 1984). It is recommended to test different eCG dosages first and then pick the one that yielded the optimum fertility. Besides the dose, the time when treatment occurs with eCG affects follicle development. For instance, the application of eCG one day after, or the last day of methallibure feeding in gilts (Polge *et al.* 1968) or 48 *versus* 24 hrs after methallibure (Brüssow & Bergfeld 1984) was associated with an increased number of stimulated follicles and also with a lower dosages of eCG needed for stimulation. Whenever a protocol includes the use of eCG it needs to be evaluated, for example by evaluating the ovary at slaughter, laparotomy, laparoscopy and ultrasound.

Besides pure eCG, different combinations of the gonadotrophins eCG and hCG have been used for stimulation of follicle development and oestrus in gilts and sows (Estienne *et al.* 2001, Knox *et al.* 2001). However, using such combinations there is evidently the risk of inducing ovarian cysts and/or of premature luteinization of follicles. The latter is assumed due to the higher LH activity of those combinations compared to pure eCG, most likely as the result of the extra hCG (Bergfeld *et al.* 1982). Increasing eCG and hCG in a combination from 400 to 700 IU and from 200 to 350 IU, respectively, was associated with an increased number of gilts with cystic ovarian degeneration (36 % *versus* 88 %) and a decrease in the pregnancy rate (50 % *versus* 65 %). In contrast, when gilts were treated with 1,000 IU eCG, only 4 % of them developed ovarian cysts (Schlegel *et al.* 1978). Moreover, if 1,000 IU eCG was given to primi- and multiparous sows 24 hrs after weaning and compared to 400 IU eCG/200 IU hCG (Suigonan®, Intervet, Unterschleissheim, Germany), the pregnancy rates, litter sizes and piglet indices were all higher in sows that received eCG only (Barbe *et al.* 1997). Taken together the majority of the studies that were conducted in former East-Germany suggest that pure eCG stimulates follicle development with better fertility results than do combinations of eCG and hCG. This is certainly the reason why standardized, lyophilized eCG preparation (e.g. Pregmagon®; IDT Dessau, Germany) is given preference in the German pig industry for the purpose of stimulating follicle development in pigs. However, in the United States, a drug that combines 400 IU eCG and 200 IE hCG, i.e. PG 600 (Intervet Canada, Guelph, Ontario, Canada) is the preferred medication, and has been shown being effective for a multitude of reproductive purposes (Kirkwood 1999).

Synthetically produced GnRH agonists have been used to stimulate follicle development in pigs, and are considered as an alternative to eCG. Recently, a synthetic analogue of lamprey GnRH-III (Peforelin; Maprelin® XP10, Veyx-Pharma, Schwarzenborn, Germany), was shown to exhibit selective FSH releasing activity in barrows (Kauffold *et al.* 2005), has been used in primi- and multiparous sows to stimulate follicle development and oestrus, and yielded similar fertility results than eCG (Engl *et al.* 2006). However, a general recommendation for its broad use would greatly benefit from further endocrine studies and supportive field results.

Induction of ovulation

Though the onset of oestrus and to some extend also ovulation can be synchronised in both gilts and sows by using eCG, the time when ovulation occurs can be still extremely variable. If fixed-time insemination is the goal, ovulation needs to be induced, either by using gonadotrophins with predominately LH activity such as hCG or by using GnRH analogues. Human CG (Hunter 1967) or equivalent substances such as pituitary extracts (Tanabe *et al.* 1949), aiming to mimic the endogenous pre-ovulatory LH-peak, were effective in gilts at inducing ovulation which occurred at approximately 40-42 hrs after treatment. If animals are treated with eCG to stimulate follicle development and then followed by hCG treatment to induce ovulation, the

interval between both treatments is however crucial to the ovulation-inducing effect of hCG. Considering that hCG should be given as close as possible to the time when the endogenous LH peak occurs in order to induce ovulation. Indeed, when this interval has been set at 78-80 hrs after eCG and the responses compared to non-treated controls, treated animals ovulated more uniformly, with ovulations occurring 42-53 hrs after hCG, whereas no control animal ovulate during this time period (Bergfeld *et al.* 1976).

Porcine LH (pLH, Lutropin-V, Bioniche Animal Health, Belleville, Canada) has been shown to be as effective as hCG at synchronising ovulation in weaned sows (Cassar *et al.* 2005, Bennett-Steward *et al.* 2008). If eCG was given the day of weaning and pLH 80 hrs later, sows ovulated between 34-42 hrs post pLH (Cassar *et al.* 2005).

After GnRH, a decapeptide, was discovered and its structure known, the pharmaceutical industry started to synthesize GnRH and thus made it available for use in swine industry. In contrast to hCG, GnRH acts at the pituitary level and stimulates the release of endogenous LH, thereby approximating what is a more "biologically normal" event than does hCG. Once more the East-German pig industry took the initiative in the development of one of the first GnRH analogues, i.e. Gn-RH vet "Berlin-Chemie" for use in swine in the late 70s. Brüssow & Bergfeld (1979) and Bergfeld & Brüssow (1979) performed intensive studies on the effect of different doses of this GnRH analogue alone or in combination with hCG on ovulation and observed that, 900 μg of this GnRH analogue alone or different combinations of GnRH/hCG (100-300 μg GnRH/100-300 IU hCG) stimulate ovulation similar to what was achieved with only hCG.

Later, another GnRH agonist (D-Phe^6-LHRH, Gonavet®, Berlin-Chemie, Berlin, Germany) was demonstrated to be even more effective at synchronising ovulation in swine than Gn-RH vet "Berlin-Chemie". As observed by repeated laparoscopy (Brüssow *et al.* 1990), in gilts treated with 50 μg D-Phe^6-LHRH started ovulating 35.5 ± 2.7 hrs after treatment and finished ovulation on average 5.9 ± 1.7 hrs later. However, animals varied in their response to D-Phe^6-LHRH, with variations being related to the interval between the GnRH injection and the LH peak, the maximum of the LH peak and the overall time needed for ovulation (Table 1). Typical LH secretion patterns observed in gilts after GnRH (Gonavet®) treatment are shown in Fig. 1.

Table 1. Effects of 50 μg D-Phe^6-LHRH (Gonavet®) on LH and ovulation in gilts (from Brüssow *et al.* 1994)

Criteria	Early* responder (n = 6)	Medium* responder (n = 6)	Late* responder (n = 4)
GnRH – LH surge (hrs)	2.2 ± 0.4^a	5.0 ± 1.0^b	9.0 ± 1.0^c
LH maximum (ng/ml)	19.6 ± 9.8^a	7.3 ± 3.2^b	3.8 ± 0.8^c
GnRH – commencement of ovulation (hrs)	35.6 ± 2.4^a	34.6 ± 2.5^a	39.6 ± 1.5^b
GnRH – completion of ovulation (hrs)	39.0 ± 1.8^a	37.2 ± 2.5^a	42.0 ± 1.5^b
LH peak - commencement of ovulation (hrs)	33.4 ± 2.5	29.4 ± 3.9	30.6 ± 0.6
LH peak – completion of ovulation (hrs)	35.8 ± 1.7^a	32.2 ± 2.7^b	35.5 ± 3.8
Duration of ovulation (hrs)	3.6 ± 2.3	2.8 ± 1.7	2.4 ± 0.2

^{a,b} $p < 0.05$,

* Animals were classified as early, medium and late responder according to their LH peak as response to GnRH.

Field trials involving a total of 2,744 gilts that were all injected with 50 μg D-Phe^6-LHRH (Gonavet®) 78-80 hrs after 1,000 IU eCG and artificially inseminated twice at fixed times, i.e. 24 and 40 hrs after GnRH, demonstrated that D-Phe^6-LHRH yields superior fertility results than if ovulation was induced with hCG. A similar observation has been made with 71,600 sows that were treated with 50 μg D-Phe^6-LHRH compared to 300 μg GnRH/ 300 IU hCG combination 55-58 hrs after eCG and artificially inseminated twice at 24 and 42 hrs after GnRH (Brüssow

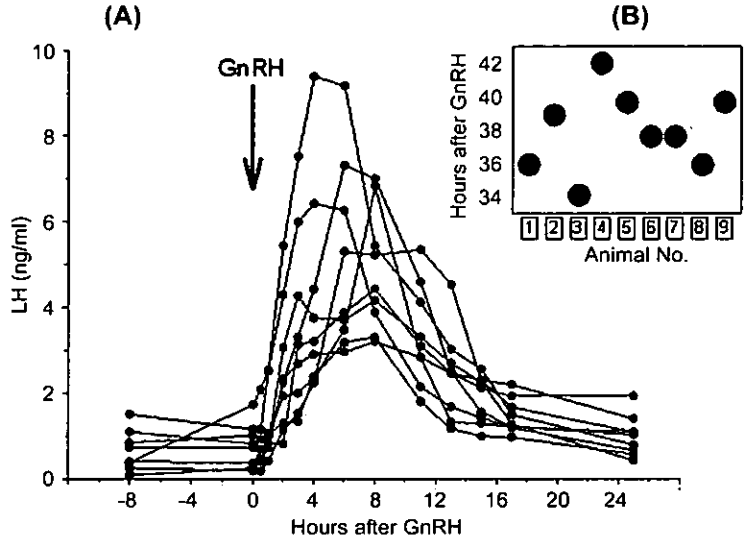

Fig. 1 LH response (A) and time of ovulation (B) in gilts (n = 9) treated with 50 µg D-Phe^6-LHRH (Gonavet®) 80 hrs after eCG

et al. 1996). Other GnRH analogues that have been tested for ovulation induction in swine are buserelin (Möller-Holtkamp *et al.* 1995), goserelin (Brüssow *et al.* 2007) and triptorelin (Taibl *et al.* 2008). All of them are effective at stimulating pre-ovulatory LH secretion in both gilts and sows. Currently however, D-Phe^6-LHRH is still the only GnRH analogue that has been licensed for use in swine in a number of European countries including Germany. The intravaginal application of GnRH containing gel (Baer & Bilkei 2004, Taibl *et al.* 2008) has been tested for the purpose of ovulation induction, but did not reach a stage beyond research. Another neuronal peptide, kisspeptin, which is a product of the KISS1 gene (Kotani *et al.* 2001) and synthesised as a preprohormone, has gained increasing interest lately since it is involved in the regulation of GnRH release, and if given to female pigs has been shown to stimulate LH release in a dose dependent manner (Lents *et al.* 2008). Though kisspeptin is thus a promising candidate to induce ovulation in pigs the question of whether or not it will ever be used in the pig industry for this purpose as part of a fixed-time insemination protocol needs further investigation.

Protocols for fixed-time ovulation and insemination in practice

The German pig industry has a long and strong history of using biotechnology in pig reproduction. Supported by continuing research efforts, the industry has many years experience with this type of technology (Hühn *et al.* 1996, Brüssow *et al.* 1996), and has thus been able to recommend different protocols that can be used for oestrus synchronisation and fixed-time insemination in pigs (Table 2). However, other approaches are also possible such as the one reported recently and performed with 3,000 sows on commercial farms in Canada (Cassar *et al.* 2005, Bennett-Steward *et al.* 2008). In these studies, sows were injected with 600 IU eCG at the day of weaning and were given 5 mg pLH 80 hrs later. They were then inseminated at fixed-times 36 and 44 hrs after pLH. Compared to untreated controls, the farrowing rate of treated sows was significantly increased (86 % *versus* 69 %).

Table 2. Treatment protocols for ovulation induction and fixed-time insemination in gilts and sows as recommended for use in practice based on experience from the East-German swine industry

Method	Gilts	Sows
Synchronisation of oestrous cycle	18 days oral application of altrenogest (20 mg/gilt/ day) (08:00 h)	Lactation until weaning
	15 days oral application of altrenogest (16 mg/gilt/day) (08:00 h)	
Stimulation of follicle development	800 – 1,000 IU eCG 24 hrs after last altrenogest (08:00 h)	*Primiparous sows* 1,000 IU eCG 24 hrs after weaning (08:00 h)
		Multiparous sows 600 – 800 IU eCG 24 hrs after weaning (08:00 h)
Induction of ovulation	GnRH* or hCG** 78-80 hrs after eCG (14:00-16:00 h)	*Lactation > 4 weeks* GnRH* or hCG** 56-58 hrs after eCG (16:00-18:00 h)
		Lactation 4 weeks GnRH* or hCG** 72 h after eCG (16:00 h)***
		Lactation 3 weeks GnRH* or hCG** 78-80 hrs after eCG (14:00-16:00 h)
1st AI	24-26 hrs after GnRH* or hCG**	24-26 hrs after GnRH* or hCG**
2nd AI	38-40 hrs after GnRH* or hCG**	40-42 hrs after GnRH* or hCG**

* e.g 50 μg Gonavet®, ** 500 IU hCG, *** weaning one day earlier in the afternoon

Conclusions

A technology is currently available that allows for manipulation of almost all key reproductive processes in the female pig including oestrus and ovulation, making fixed-time insemination possible. Batch farrowing systems may profit from using this technology due, at least in part, to savings on labour and production of large batches of uniformly developed and healthy pigs. This technology does however not allow for compensation of health problems and/or mismanagement. Understanding of reproductive processes and the availability of hormonally active drugs are essential requirements when deciding to use this technology. However, several factors related to the farms themselves, as well as to the drugs may influence the effectiveness and outcome of this technology. It is thus recommended to adapt this technology to each individual farm.

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