

Objectives of Assisted Reproductive Technology

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■ **Increasing Lean Meat Output per Boar Space per Annum**

Artificial insemination is a significant genetic tool. In a two service, mating regime a single insemination represents in excess of 500kg of pig meat. The genetic and financial differential between those carcasses derived from high specification AI boars and those produced from Natural Service is significant. This of course assumes that the boars from which the semen is disseminated are purchased on the basis of genetic merit, have been screened objectively (ALOKA / CAT scanned), biometrically assessed and diagnostically tested for specific gene markers. The meat value differential and benefits of improved growth performance alone, attained from AI derived carcasses has been equated to C\$2.09 per pig (Cosgrove, 1996), which extrapolates to >\$10 per single insemination. Additional advantages in costs of boar purchase and maintenance (\$5-13 per bred female), in labour costs derived from a more efficient breeding process, and increased herd bio-security were also presented as major advantages of using AI technology.

The concept of significantly increasing the lean meat output per AI boar at stud through the practical application of assisted reproductive technology is of considerable importance to vertically integrated businesses.

The notes below form the basis of a plan to effectively double the output of lean mass per boar space without impairing fertility or fecundity.

Through an initial series of applied experiments, a simple service system to ensure good oestrus detection, enhanced oestrus behaviour and artificial insemination practice that improved sperm transport was developed. This system has improved fertility, more importantly it has standardised practice.

From this base new extender technology was used and the Deep Golden Pig (catheter) was developed in order to reduce sperm concentration.

■ **Improving Oestrus Behaviour and Oestrus Detection as the Basis of Good AI Practice**

The exponential increase of artificial insemination in swine has unfortunately not been matched by an increase in research on boar-sow interactions to optimise sexual stimulation before, during or after insemination. All too often, disparate fertility levels occur in units practicing artificial insemination. Good heat detection is fundamental to ensure that the sow is at the right stage of oestrus at the time of artificial insemination, but often producers who have substantially reduced boar to sow ratios fail to utilise this depleted "boar power" efficiently.

There have been numerous studies of the effects of specific boar stimuli (visual, olfactory, auditory and tactile) on the attainment of puberty (Severinck et al., 1997; Hughes et al., 1990), but only a few studies have incorporated observations on the duration, and initial expression of oestrus in sows and gilts. An increase in the amount of boar stimulation during oestrus detection increases the expression of oestrus of sows. Jongman et al. (1996) even showed that stimulation by more than one boar (oestrus detection in a detection mating area where the sows are surrounded by several boars) reduced the weaning to oestrus interval and increased the duration of oestrus by half a day. Although these improvements could be related to the "primer" effect of boar pheromones, the results could also be explained as the result of increased efficacy of oestrus detection because of increased provision of sexual stimuli at the time of heat detection. Between boars, variability in stimulation value (olfactoral stimuli) affect both proceptive and receptive behaviour (Hughes et al., 1985), and under multi-sire conditions, there was a certain partner preference (Tanida et al., 1991). Conversely, continual contact with boars has an adverse effect on oestrus expression, as gilts habituate to boar "signalling" stimuli (Hemsworth et al., 1988). Dyck (1988) found a similar effect in post-weaned sows.

Integration of incoming stimuli and the basic interpretation of the sensory signals by the individual occur through known physiological mechanisms (Hemsworth et al., 1997). For instance, visual and olfactory stimuli induce production of neuropeptides, hormones and pheromones, which in turn induce biological and behavioural responses to the original stimuli provided. Therefore, olfactory, auditory, visual and tactile stimuli are the essential and irreplaceable precursors of good courtship behaviour and ethological complexes in males and females. Part of the influence of boar stimulation on the onset and duration of oestrus may be explained by an involvement of prolactin, since Prunier et al.

(1987) found that every introduction of a boar during oestrus causes a release in prolactin. Also, Van de Wiel et al. (1981) found that the onset and duration of oestrus was positively associated with concentrations of prolactin.

Reducing the ratio of boars to sows obviously reduces the opportunity for pre-insemination stimulation well below levels experienced when all sows are served naturally; however, it is inappropriate to blame poor heat detection per se for variable fertility. It is more a product of poor sexual stimulation and an unfortunate lack of respect for the behavioural/physiological sequence of events that naturally occurs between boar and sow, and culminates in attainment of lordosis or standing heat. More importantly, there is often an inability to measure this lordosis response objectively by the stockman. Simply, if there are no sexual stimuli there will be reduced accuracy of heat detection or no display of standing oestrus. The stockperson controls the degree of sexual stimuli provided by the boar to the sows, and thus the chance to take advantage of the pre-cursors of the behavioural/physiological mechanisms within the sow that induce good oestrous behaviour. Full expression of oestrus can be achieved by allowing good olfactory, tactile, auditory and visual stimuli from a boar placed in front of the sow. Conversely, the stockperson can negate the benefits of sexual stimuli by impeding effective interactions between the boar and sow, simply because of a lack of awareness of the significance of providing adequate stimulation. It could be that by failing to open a gate to allow the boar to interact and “chat” to sows at artificial insemination the stockperson is markedly reducing the fertility achievable with artificial insemination.

It is essential that behavioural patterns are observed/recorded in an objective and quantitative manner, and that their significance is understood to match them as closely as possible to the environmental conditions available to enhance reproductive performance. Conversely, pig producers must design and build breeding facilities which allow well ‘managed’ social / sexual interaction between male and female. The immediate farm/service house environment and associated management practices may impinge upon important components of the social environment, culminating in disrupted behavioural sequences and reduced physiological sexual impetus at and around insemination.

Low reproductive rates of herds may be partly indicative of the effects of chronic stress, although obviously other factors such as social facilitation and nutrition cannot be scientifically discounted. Minimising the influences of chronic and acute stress upon reproductive efficiency is intrinsic to achieving reproductive efficiency. However, many sow herds achieve high pregnancy rates (>90% conception), indicating that sows have a robust physiology and generally adequate habituation to their immediate environment.

In summary, therefore, artificial insemination will remain wholly artificial unless “full range” sexual stimuli are provided through insemination. Initially, standing

oestrus needs to be objectively defined; is she in heat, yes or no? However, given the discussion above, the question “Is she in heat?” should really become, “Has she enough stimulation to display heat?” Yes, or no? Provide these “full range” stimuli first, then ask “Is she in heat?” second.

■ Improving Sperm Transport

Other physiological mechanisms, such as oxytocin release, essential for myometrial contraction in the uterus and efficient sperm transport in the sow, also require switching on biologically through the provision of appropriate stimuli. These responses are also critical for achieving maximal fertility and are equally dependent of effective use of the boar at the time of insemination.

Recent Studies on Improved Breeding Management Regimens

One recent study investigated the effect of “segregated service management” (SSM). This technique involves the complete tactile segregation of boars from sows within the service area from the point of weaning to heat detection / service, with the intention of maximizing the opportunity to properly detect the onset of heat. Sows cannot see, hear or touch boars for the initial four days post-weaning. Subsequently, boars were only allowed to provide stimuli to the sows at the time of heat detection or insemination, and only when stock-people were present. This technique substantially improved conception rate (Table 1), as a result of focusing boar-sow interactions to the critical breeding period. It appears that utilising all of the important boar stimuli in a concentrated burst, at the time that the specific task of heat detection is carried out, helped to clearly define the critical lordosis period.

Table 1. Mean reproductive results from a comparison of Control and SSM breeding strategies.

Performance	Control	SSM
Total services performed	1196	1193
% bred in < 7 days	94.5	93.2
Farrowing rate (%)	86.2 [*]	90.1 [*]
Total born per litter	11.40 ^{**}	11.74 ^{**}

*p<0.01; **p<0.05

The significant improvement in Conception Rate and Litter Size indicates the effectiveness of the SSM regimen.

Following on from the initial trial, additional work utilising Real Time Radiography has demonstrated improved sperm transport with SSM treated sows, when compared with sows that had no boar contact or continual boar contact during the heat detection/breeding period. It is the author's intention to repeat the time sequencing with CAT scanner technology that will enable a more accurate measurement of the time taken for semen to reach the oviduct post-insemination.

Reduction of Sperm Concentration.

During oestrous, sperm cells are deposited by artificial or natural insemination at the utero-cervical junction and are mainly transported to the utero-tubal junction as a result of the contractile activity of the uterus (Einarsson, 1985; Kemp & Soede, 1997). The specific stimulus provoking ejaculation in the boar is the locking of the glans penis in the cervical ridges of an oestrous sow. Due to the influence of ovarian steroid hormones, these muscular ridges become taut and oedematous at oestrus, and their arrangement is such that the lumen of the cervix tapers towards the internal os, enabling the glans penis to be gripped by the female tissues. During thrusting of the penis through the anterior vagina and into the cervical canal, there is also an anti-clockwise twisting component to the penile shaft, causing the spiral folds of the glans to interdigitate tightly with the cervical wall. The pressure derived from this lock leads to ejaculation, a situation which is mimicked during manual collection of boar semen and during the use of the "Melrose" catheters first developed for AI use in pigs. The foam tip of the more recently developed insemination catheters also locks and interdigitates tightly with the cervical wall during the process of artificial insemination and placement of the insemination catheter.

Spermatozoa are deposited within the cervix, and despite insemination of large quantities only a relatively small number reach the oviducts (First *et al.*, 1968; Hunter, 1996). Sperm cells have entered the oviduct within 15-30 minutes after insemination or mating to form a sperm reservoir in the caudal 1-2 cm of the oviductal isthmus (Hunter, 1984). This region of the oviductal isthmus has favourable storage conditions for sperm cells and regulates the release of capacitated, activated sperm cells to the site of fertilisation above the ampullary-isthmic junction of the oviduct.

The uterus is a hostile environment for sperm cells. The motility of sperm cells in the uterus decreases quickly after insemination and many sperm cells are removed from the uterus within a few hours after insemination by back flow and phagocytosis (Baker *et al.*, 1968; Viring and Einarsson, 1980). Within 2 hours of insemination, approximately one-third of inseminated sperm cells were found in back flow from the vagina (Einarsson, 1981). Of the remainder, most is

absorbed by the uterine mucosa within 5 hours. Knox (2001 - unpublished, personal communication) has recorded in excess of 80% sperm cell loss from the vagina of sows up to 24 hours of insemination similar loss has been reported (Peltoniemi et al., 2000).

The rationale for the development of the Deep Golden Pig from a reproductive standpoint was to enable significant reduction in sperm concentration per insemination by placing smaller numbers of sperm into a post-cervical (uterine) location. Other recent studies have investigated the use of these normally sub-optimal sperm concentrations in situations of current applied AI practice at farm level.

Artificial insemination (AI) in pigs requires 2 to 3 billion spermatozoa to achieve consistently high fertility with current practice of inseminating into the posterior region of the cervix. We have investigated the potential advantages of inseminating through the cervix into the caudal region of the uterus using lower sperm numbers. Total sperm doses from 22 boars of 3, 2 or 1 billion spermatozoa were packaged in 80ml volumes in X-Cell® extender in gene-flat-pack bags®. A novel insemination device, the Deep Golden Pig™, was employed which permits the ready introduction of a narrow catheter through the cervix into the uterus without traumatic injury to the mucosa. This was compared with the standard Golden Pig® device that allows semen to be deposited in the posterior folds of the cervix. Sows of two different genotypes and of parities ranging from 2 to 11 were used. They were selected solely on the basis of a weaning to oestrus interval of 4 to 6 days. Two inseminations, with a 24h interval between them, were given to each sow. Pregnancy was determined at 35 d by ultrasound scan, and farrowing and litter size recorded. Pregnancy and farrowing data were very similar. The standard inseminating device produced farrowing rates of 91.1, 91.8 and 65.8% for insemination with 3, 2 and 1 billion spermatozoa, whereas the deep insemination device gave rates of 90.5, 90.5 and 86.9%. Only the 1 billion dose with the standard device was significantly different from the high dose control ($P < 0.001$). Similarly, the mean litter sizes with the standard device were 12.5, 12.6 and 10.3 and with the deep insemination device 12.3, 12.3 and 12.1. Only the 1 billion sperm dose with the standard device was significantly lower ($P < 0.001$). None of the covariates differed significantly and there were no significant interactions with treatment. We conclude that trans-cervical insemination in the sow is simple, effective and safe, and allows the sperm dose to be reduced to 1 billion spermatozoa.

From a scientific stand-point this trial indicated that utilisation of 1 billion sperm cells and the Deep Golden Pig led to a farrowing rate decrease of <5% and a LS decrease of <0.5 piglets per litter when compared to other more conventional treatments. Statistical analysis of the data has shown no significant difference. Producers will however have calculated significant

monetary loss. To clarify the concept of the DGP it is essential that we understand the following points.

- The objective of the trial was not to demonstrate that improved fertility could be obtained with the DGP at 2 or 3 billion sperm cells per single insemination against existing technologies (Golden Pig). The objective of the work was to demonstrate that fertility and fecundity were achievable at 1 billion sperm cells with a non-surgical AI technique, and that fertility and fecundity were not significantly impaired as a result of DGP utilisation.
- The trial differentiates the DGP as a 'safe' non-surgical method of trans-cervical or intra-uterine semen deposition irrespective of sperm cell concentration >1 billion total sperm cells per insemination. Extensive histological examination of reproductive tracts indicated no tissue damage post-DGP insemination.
- Vasquez (2000) & Rath et al. (2000) have also been successful at reduced sperm concentration albeit through hormonal manipulation and ovulation synchrony. It was the intention of IMV however that the DGP must be easily utilisable by service personnel as standard farm practice, irrespective of service regimen.

Table 2. A comparison of fertility and fecundity of sows after artificial insemination twice in a single oestrus with a standard device (Golden Pig, control) and a novel deep inseminating device (Deep Golden Pig).

Device	Sperm dose (billions)	% Pregnancy	Farrowing (%)	Litter size (n)	Number born live (n)
Golden Pig	1	66.2	65.8	10.3	9.0
	2	91.1	91.8	12.6	10.9
	3	91.3	91.1	12.5	10.9
Deep Golden Pig	1	88.7	86.9	12.1	10.9
	2	92.6	92.5	12.3	10.8
	3	91.8	90.5	12.3	11.0
Total N		3230	3201	2768	2768

Table 3. Comparison of fertility data after deep (intrauterine) insemination with that in the next parity with conventional insemination with 2×10^9 spermatozoa (n=1153).

Insemination treatment	Average parity	Farrowing %	Litter size	Farrowing index (days)
Intrauterine	3.7	91.1	12.31	152.3
Conventional	4.6	90.7	12.57	152.4

■ **Reduced Volume / Refrigerated Semen**

The discussion above reflects current literature in relation to a number of projects on the improvement of AI technology for which data are presented. Several additional ongoing studies attempt to further investigate utilising assisted reproductive technology as a means of increasing lean mass output per boar space per annum. Although the reduced semen dose volume trial reported above is incomplete, the concept of significantly reducing volume of semen doses has several advantages in achieving the goal of rapidly increasing lean mass production per available boar space. The refrigeration of semen to extend semen viability is the subject of several concurrent investigations, particularly at an intracellular level. However, commercial field trials have been undertaken and recent data from this study will be presented at the Banff Pork Seminar to illustrate the potential to increase AI effectiveness in the future.

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