Fast genetics™
TECH EXTENSION

How low can we go?

Finding a practical path to produce more pigs from fewer and better boars

www.fastgenetics.com

Artificial insemination (AI) has become the standard breeding method for dairy cattle, poultry, and swine producers largely for the same principle reason. Dividing the semen of a high genetic merit male across as many females as possible increases the number of offspring he influences, and the rate of genetic change achieved compared to natural mating. Since AI became widespread in North American swine herds in the 1990's, improvements in several areas of the AI process have allowed gradual reduction of the number of sperm required per gilt or sow in estrus.

Industry averages for sperm per AI dose and the number of AIs per female have likely decreased from 5 to 2.5 billion total sperm and from 3 to 2 AIs (Flowers and Esbenshade, 1993; Knox, 2016). This 66% reduction (15 vs 5 billion sperm/female) of sperm per female since we started using AI to replace natural service has helped capture additional genetic value.

Even more efficiency can be gained if we can continue to find practical methods to better utilize the sperm of superior genetic merit boars without compromising sow reproductive performance. Fast Genetics has an active research program to develop such methods to more efficiently use boar sperm and a vested interest to open a path to large scale implementation of sex sorted semen.

Successful fertilization in pigs, and other animals, can be thought of as an interaction between what, when, and where. Sperm, and their quantity and quality are the what; synchrony of sperm introduction with release of the eggs (ie, ovulation) is the when; and site of semen deposition in the sow's reproductive tract is the where. As long as there are no major deficiencies in any of these three areas, the percentage of eggs fertilized (ie, fertilization rate) tends to be high (eg, >90%) in swine. Sperm seem to be able to maintain their fertilizing capability in utero for longer than ovulated eggs can maintain developmental competence (eg, ~24 vs 8 h; Hunter, 1967; 1990). In addition, the standing estrus or "heat" that we use to determine when gilts and sows are receptive and fertile can be quite long (eg, > 48 to 72 h) with ovulation occurring at about 66% of its duration (eg, > 32 to 48 h after onset of estrus; Soede and Kemp, 1997). Therefore, applying at least two AIs separated by 24 h, soon after onset of estrus, has been an effective strategy to get a viable population of sperm in place and waiting for ovulation to occur.

> *"Successful fertilization can be thought of as an interaction between what, when, and where."*

What: Sperm and its quantity

Despite the longer lifespan of sperm in utero compared to eggs in the oviduct, the fertility (ie, conception and farrowing rate) and (or) fecundity (ie, litter size) of sows inseminated multiple times based on estrus can be reduced when sperm per AI dose reaches an inadequate level such as 1 billion (Watson and Behan, 2002; Rozeboom et al., 2004). We wanted to know when sperm per AI dose would become insufficient and reduce fertility and fecundity if AI-to-ovulation interval variation was limited by hormone synchronization combined with a single fixed-time AI (SFTAI).

In a field study, groups of Fast York H sows at a depopulating farm were synchronized with a GnRH agonist post-weaning and administered a SFTAI regardless of standing estrus containing either 1.2, 0.6, 0.3, 0.15, or 0.075 billion total sperm. Three Fast York A boars with good semen quality at a nearby stud were collected for each sow wean group and their pooled semen divided to make all AI doses in an effort to limit semen age (1.5 d) and any boar fertility effects. Sows were slaughtered 27 d after AI and their reproductive tracts were recovered to confirm pregnancy and count embryos to estimate litter size.

Number of sperm per AI dose had a substantial effect on number of embryos but not pregnancy rate (Table 1). Pregnancy rate was relatively stable until the lowest 75 million sperm per AI dose level. In contrast, number of embryos exhibited a stepwise decrease of 1 embryo from the 1.2 billion sperm per AI dose level down each time sperm numbers were halved. The product of pregnancy rate times number of embryos in the last row of Table 1 puts embryos on a per sow AIed basis by accounting for open sows (ie, zero embryos).

	Sperm/Dose, Billions				
	1.2	0.6	0.3	0.15	0.075
Sows	67	136	133	133	64
Preg, %	84.1	80.7	82.9	80.6	69.1
Embryos	14.3	12.9	11.2	10.3	9.6
Embryos/Sow AIed	12.0	10.4	9.3	8.3	6.6

Table 1. Effect of number of sperm per SFTAI dose on pregnancy rate and number of viable embryos of sows at 27 d after insemination. Fast Genetics unpublished data.

When: Synchrony - insemination and ovulation timing

Previous work with fresh semen and moderate 2 to 3 billion sperm per AI doses has suggested that as long as one insemination occurs within 24 h to 0 h window prior to ovulation, high fertilization rates (eg, 90%) can be achieved (Kemp and Soede, 1997). We wanted to know if these limits would remain the same at lower sperm per AI dose levels and if insemination closer to ovulation could make up for insufficient numbers of sperm.

Through repeated ultrasound examination of follicles every 8 h in this field study, we were able to estimate the time of ovulation which averaged 16 h after SFTAI. Only 11% of the sows were inseminated outside the ideal 24 to 0 h prior to ovulation window (Table 2).

AI-to-ovulation interval had a large effect on pregnancy rate but not number of embryos. Insemination after ovulation resulted in reduced pregnancy rate as expected but only 8 sows had such synchrony (ie, “early” ovulation). Insemination > 24 h before ovulation yielded the lowest number of embryos and only 42 sows had such long AI-to-ovulation intervals (ie, “late” ovulation).

Even though there is a numerical pattern for increasing embryos with shorter AI-to-ovulation interval classes, this effect was not significant. There was also no interaction between the numbers of sperm per AI dose and pregnancy rate or number of embryos. It appears that tighter synchrony propped up number of embryos at these limiting sperm per AI doses but that it had the opposite effect on conception.

Fast Genetics™

	AI-to-Ovulation Interval, h				
	32 to 24	24 to 16	16 to 8	8 to 0	0 to -8
Sows	42	259	121	30	8
Preg, %	81.6	89.1	81.4	76.8	51.4
Embryos	10.9	11.7	11.9	12.8	16.5
Embryos/Sow AIed	8.8	10.3	9.5	9.8	8.2

Table 2. Effect of SFTAI-to-ovulation interval on pregnancy rate and number of viable embryos of sows at 27 d after insemination. Fast Genetics unpublished data.

Where: Site - Semen Deposition Technique

Conventional swine AI originally deposited semen into the sow's cervix mimicking natural mating. More recently, the use of a smaller diameter secondary catheter, passed through a primary conventional catheter, to transverse the cervix and deposit semen in the uterine body (aka, post-cervical AI or intrauterine insemination, IUI) has become almost standard practice.

Reduction of sperm wastage and increased reproductive performance at reduced sperm doses are purported advantages of IUI over cervical AI (Watson and Behan, 2002). Such deeper is better thinking has even led to the development and testing of longer, flexible, secondary catheters that can be advanced past the uterine body and two-thirds of the way up a uterine horn to deposit semen (deep IUI or DIUI; Martinez et al., 2001; 2002; Mozo-Martín et al., 2012). However, since no published studies have compared IUI versus DIUI sites of semen deposition (ie, body vs horn) at the same sperm per dose, it has not been possible to determine if deeper uterine semen deposition is actually more effective at maintaining reproductive performance at limiting numbers of sperm per dose.

In this same field study, we compared a commercially available IUI catheter and a Fast Genetics proprietary DIUI catheter at three sperm per AI dose levels (Table 3). Reducing the number of sperm per SFTAI dose caused a significant and similar decrease of number of embryos at both semen deposition sites. Deeper uterine horn deposition of semen compared to IUI did not ameliorate this effect of insufficient sperm.

Pregnancy rate was not significantly affected by sperm per AI dose or site of semen deposition though it did tend to be reduced at the 0.15 billion sperm per AI dose level for IUI compared to DIUI sows. There was also no sperm per AI dose by site of semen deposition interaction for pregnancy rate though the numbers do appear to trend in opposite directions.

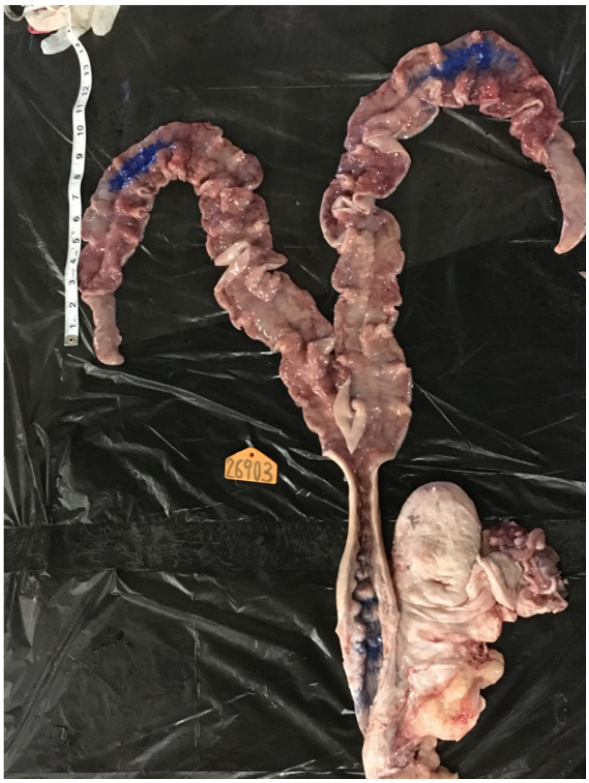

Figure 1. Example of site of deposition verification (blue stain) of a bicornuate deep intrauterine insemination catheter designed to go into both the left and right uterine horn.

Overall, IUI did not fail to generate similar pregnancy rates and embryo numbers compared to DIUI which was somewhat unexpected. Since DIUI was only into one uterine horn (ie, left or right) it could be increasing fertilization rate at one oviduct at the expense of the other as some have reported an imbalance (Martinez et al, 2006; Buranaamnuay et al., 2011). Bicornuate DIUI, into both uterine horns, remains to be tested and is an active area of Fast Genetics research and development (Figure 1).

	Intrauterine Site of Deposition					
	IUI (Body) Sperm/Dose, Billions			DIUI (Horn) Sperm/Dose, Billions		
	0.6	0.3	0.15	0.6	0.3	0.15
Sows	70	62	68	66	71	65
Preg, %	82.3	79.0	74.1	78.2	83.3	85.9
Embryos	13.3	11.4	10.1	12.7	11.3	10.7
Embryos/Sow AIed	10.9	9.0	7.5	9.9	9.4	9.2

Table 3. Effect of SFTAI with an intrauterine versus deep intrauterine insemination catheter and sperm per AI dose on pregnancy rate and number of viable embryos of sows at 27 d after insemination. Fast Genetics unpublished data.

Summary

Among the three S's of fertilization, sperm quantity was the largest factor in determining the level of reproductive success with litter size being more sensitive to limiting sperm numbers than pregnancy rate. Reduced AI-to-ovulation intervals and deeper uterine deposition of semen were not able to curb the negative reproductive effects of insufficient sperm. Non-surgical bicornuate deposition of semen in each uterine horn remains to be tested and may or may not be advantageous since surgically depositing sperm even deeper at the UTJs and(or) in the oviducts seems to be required to substantially reduce the numbers of sperm required. Practical methods to further reduce sperm per female will continue to open access to higher genetic merit boars, decrease genetic lag, and will add even more value by making technologies such as sex sorted sperm implementable on a larger scale.

Dr. Brad Belstra
Manager of Reproductive Services
Fast Genetics

References available upon request