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Improved Semen Characteristics in Boars Selected for Testis Size

**Ying-Tsorn Huang
Rodger Johnson¹**

The practice of artificial insemination is increasing in swine herds. There is considerable variation among boars in age when semen can be collected, volume of semen, and sperm concentration, motility of sperm cells, and frequency of abnormalities in semen. Selection of boars has been for performance traits such as growth rate and backfat thickness and female reproductive traits such as size and weight of litters of dams and other female relatives.

Little selection for male reproductive traits has been practiced. However, selection practices that result in boars with greater quantities of high quality semen would improve the efficiency of artificial insemination and be beneficial to the industry.

Testis size is correlated with daily sperm production and with total sperm numbers in the epididymis in several species, including swine. At Nebraska, an experiment was conducted in which selection for increased weight of testes at 150 days of age (TS line) was practiced. A randomly selected line (control, C line) was also maintained for 10 generations. The purpose of this article is to report results of an experiment which evaluated differences in quantity and quality of semen produced by boars of the TS and C lines.

Semen was collected from boars 3 times per week or daily when boars were between the ages of 8 and 13 months.

Materials and Methods

The selection experiment and re-

sponses in weight of testes and in body weights and backfat depths of boars and gilts after 6 generations of selection were described in the 1990 Nebraska Swine Report. Therefore, only a brief description of the selection experiment will be given here.

The population was a Large White-Landrace composite. In the base generation littermates were randomly assigned to the TS and C lines. Thereafter, lines were closed - all replacements in each line were selected from within the line. Lines were maintained with 40 to 45 litters by 15 sires each generation, and the generation interval was one year.

At 140 and 160 days of age, width and length of paired testes were measured with a calipers and these measurements were used to predict weight of testes at 150 days of age (PWT). In line TS, all males were left intact. The 15 boars with the greatest PWT were selected each generation. At least one gilt was randomly selected from each litter. In line C, one boar was selected randomly from each half-sib family, and at least one gilt was selected randomly from each litter.

There were two replications of the experiment reported herein that were done in two years. In year 1, 6 C and 14 TS boars from the 10th generation were used and in year 2, 12 C and 11 TS boars from the 11th generation were used. These boars were selected randomly from within half sib families. Therefore, each boar had a different sire and dam.

When boars were between 7.5 and 8 months of age, they were transported from the experimental herd at the Agricultural Research and Development Center, Mead, NE, to the Animal Sci-

ence Building, Lincoln, NE. They were individually penned in a room in which there was a semen collection area (2.5 x 2 meters) with a dummy and an area for processing semen. During the experiment boars were fed 2.5 kg per day of a diet formulated to contain 14% crude protein. Temperature was maintained at approximately 20° C.

Within four weeks of arrival, all boars had been trained to mount the dummy and their semen could be collected. Then they were placed on a schedule of three collections per week for three weeks followed by daily collections for three weeks. At three collections per week semen was collected from each boar on Mondays, Wednesdays and Fridays or on Tuesdays, Thursdays and Saturdays. After the period of daily collections, semen was collected once per week for five weeks from each boar. A five-week rest period was used because the length of the cycle of the seminiferous epithelium, the interval of time for one complete series of cellular associations to appear within the seminiferous tubules, is approximately 39 days in the boar. After the rest period, semen was again collected three times per week followed by daily collections for three weeks.

When semen was collected, the times from when boars entered the collection area to when they mounted the dummy and from when the penis was gripped to when the ejaculation was completed were recorded. Volume of the sperm-rich fraction of the semen was recorded. The sperm-rich fraction is the second part of the ejaculate. It is the viscous-chalky, milky-white portion that contains 80 to 90% of the total spermatozoa. Semen samples were used

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to determine percentage of motile sperm, percentage of abnormal sperm, concentration of sperm cells and total number of sperm cells per ejaculate.

Sperm motility was determined subjectively by observing sperm cells in undiluted semen under a microscope (100 x). After properly staining, morphology of sperm cells was determined by observing 10 sperm cells in each of 10 different microscopic fields. Sperm cells with piriform head, tapering head, narrow head, small head, giant head, short wide head, coiled tail, abnormal attachment of the midpiece, distal plasmic droplet, proximal plasmic droplet, no tails, and double head were classified as abnormal and expressed as a percentage. A spectrophotometer was used to determine concentration of sperm cells and total number of cells was determined by multiplying concentration times volume.

After the last of the 64 semen collections taken from each boar, the boar was castrated and the right epididymis and testis were separated and weighed. Three samples of one gram each from the proximal, mid, and distal regions of the testis were used to determine daily sperm production. The number of mature sperm cells in the cauda region of the epididymis was determined. The tissue was homogenized and the number of homogenization resistant sperm nuclei was counted in duplicate for each sample with a hemacytometer.

Results

At the 10th generation, PWT for line TS boars averaged 555.7 g compared to 337.4 g for C boars. The average response per generation was 19 g, 5.5% of the base generation mean. The trait directly selected for, PWT, had a large variance - the within line - generation standard deviation (SD) ranged from 59.2 to 95.9 g. Corresponding coefficients of variation (100 x SD/mean) ranged from 12 to 28%. There was a large amount of phenotypic variation in PWT, consequently selection differentials were large. The realized heritability was $.35 \pm .02$, therefore the genetic variance in PWT also was large.

Even though selection was practiced in only one sex, the response was approximately twice what normally occurs when selection is for most production or reproduction traits.

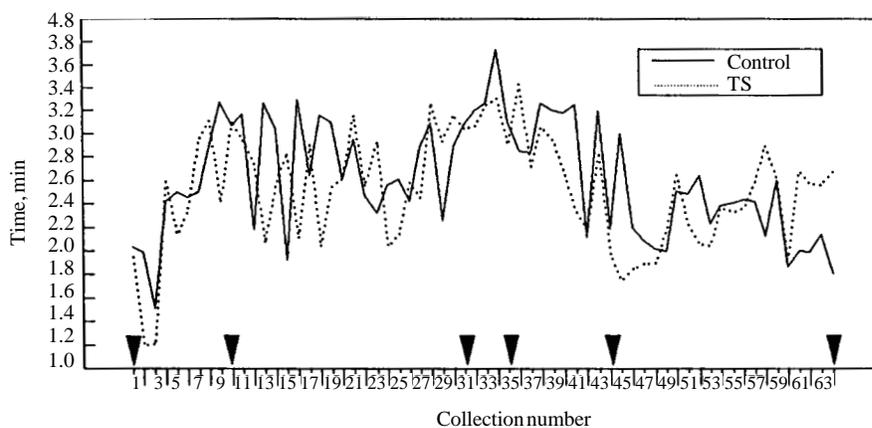
Because the direct response to selection was so large, the lines are an excellent resource to measure responses in correlated traits. It is generally thought that selection for increased size of testes will increase reproductive characteristics of males. The remainder of this paper describes the effects of this selection on semen characteristics of boars.

Data from three collections per week and daily collections were analyzed separately because variances for several traits were different for these two treatments. Within each treatment, analyses were done to determine if there were line x period (period 1 was collections at the younger age, and period 2 the second sequence of collections at the older age) and line x collection number and line x period x collection number interactions. Line x period interactions were significant for several traits, but line x collection number and

line x period x collection number were not significant for any trait. Therefore, results are presented graphically to illustrate responses over time, and means for the lines were compared within each period separately for three collections per week and daily collections.

Time to mount the dummy at each collection and results of comparisons of line means are shown in Figure 1. This time increased linearly during the first three weeks of three collections per week, remained flat during the first three-week period of daily collections through the rest period, and then declined linearly during the second three-week period of three collections per week and again remained flat during the last period of daily collections.

TS boars took .4 minutes less ($P < .05$) to mount the dummy during the first period of daily collections, otherwise lines did not differ. During three collections per week, average time to mount the dummy was 2.4 minutes at the younger age and 3.1 minutes at the older age ($P < .01$). However, during periods of daily collections, the average time was 3 minutes at the younger age

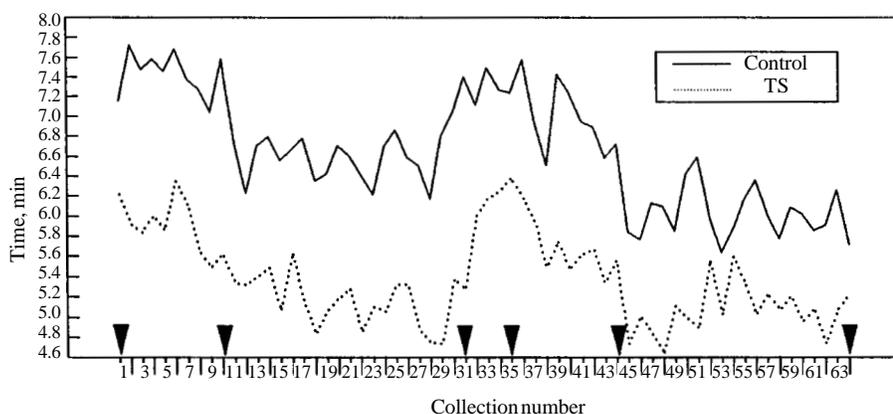


Number 1-9 & 35-43, 3 times/wk; Number 10-30 & 44-64, daily; Number 31-34, 1 time/wk

	P1(3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2(3 times/wk)	P2 (Daily)
Control	2.5	3.1	3.3	3.2	2.2
TS	2.2	2.7*	3.2	3.0	2.2

**Means differ, $P < .01$. *Means differ, $P < .05$.

Figure 1. Time to Mount the Dummy



Number 1-9 & 35-43, 3 times/wk; Number 10-30 & 44-64, daily; Number 31-34, 1 time/wk

	P1(3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2(3 times/wk)	P2 (Daily)
Control	7.5	6.7	7.3	6.8	5.9
TS	5.9**	5.2**	5.9**	5.7**	5.0**

**Means differ, $P < .01$. **Means differ, $P < .05$.

Figure 2. Time to Complete the Ejaculation

and 2.3 minutes at the older age. There is no obvious explanation for this interaction.

Time to complete the ejaculation is illustrated in Figure 2. No interactions were found for this trait. TS boars took from .9 to 1.6 minutes less ($P < .01$) to complete the ejaculation than C boars.

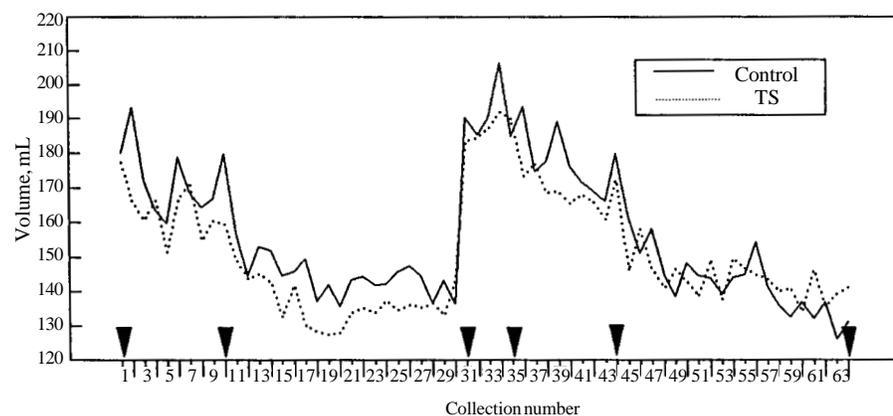
There was a gradual, linear decline over collections until the rest period in both TS and C boars, when average time increased, followed again by a decline until the end of the experiment. At three collections per week, means for younger and older boars were 6.7 and 6.3 minutes ($P < .01$), respectively.

During periods of daily collections, younger boars averaged 5.9 minutes and older boars 5.5 minutes ($P < .01$) to complete the ejaculation.

Volume of semen per ejaculate declined linearly during the first period of three collections per week and reached a lower plateau during the first period of daily collections (Figure 3). Volume quickly increased during the rest period to an amount greater than at the beginning of the experiment. The linear decrease in volume during the second period of three collections per week was similar to that in the first period. During the second period of daily collections volume declined further, but the rate of decline was less. The interaction of line x period was not significant. Volume of semen for TS boars was consistently less than for controls, and line differences were significant during each period except the second period of three collections per week. Within treatment, volume was greater (approximately 3 mL) for older than younger boars, but these differences were not significant.

Percentage of motile sperm decreased linearly during both periods of three collections per week, but the rate of decline was relatively small (Figure 4). During daily collections at the younger age, the rate of decline in motility was quadratic. The decline was sharp for the first 7 days, and then a lower plateau was reached. The response was similar at the older age during daily collections except the early rate of decline was less steep and the lower plateau was at a greater value. Lines responded similarly over time. They also did not differ during periods of three collections per week, but percentage motility was greater ($P < .01$) for TS boars during periods of daily collections. At three collections per week, percentage motility did not differ between younger and older boars, but at daily collections, motility averaged 73.8% for boars at the younger age and 77.6% ($P < .01$) at the older age.

Percentage of total abnormal sperm cells increased during the experiment



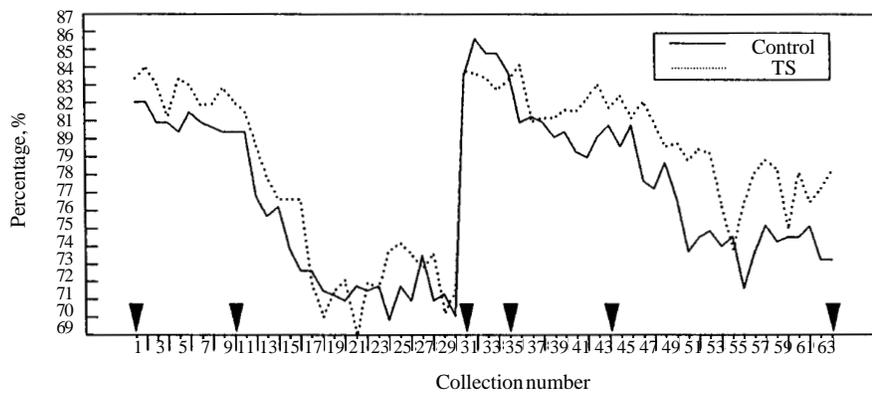
Number 1-9 & 35-43, 3 times/wk; Number 10-30 & 44-64, daily; Number 31-34, 1 time/wk

	P1(3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2(3 times/wk)	P2 (Daily)
Control	173.6	145.3	193.1	173.5	141.3
TS	163.4*	138.3**	186.9	170.3	145.6*

**Means differ, $P < .01$. **Means differ, $P < .05$.

Figure 3. Volume of Sperm-rich Fraction

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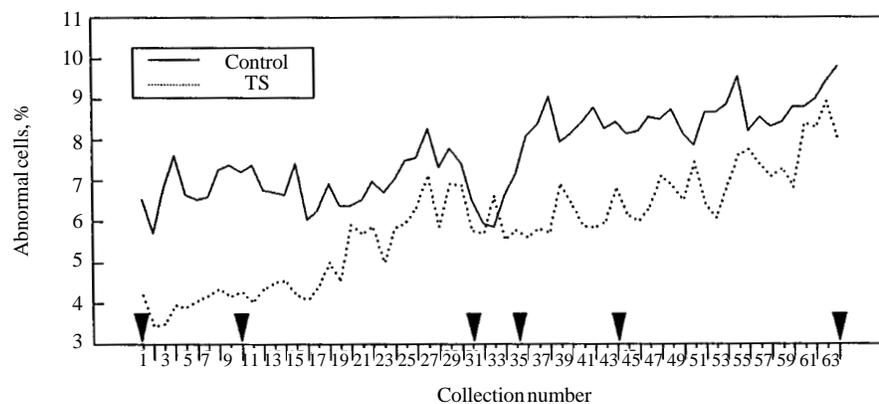


Number 1-9 & 35-43, 3 times/wk; Number 10-30 & 44-64, daily; Number 31-34, 1 time/wk

	P1(3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2(3 times/wk)	P2 (Daily)
Control	82.0	72.8	84.7	81	76.7
TS	82.7	74.8**	83.4	82	78.9**

**Means differ, $P < .01$. **Means differ, $P < .05$.

Figure 4. Percentage of Motile Sperm Cells



Number 1-9 & 35-43, 3 times/wk; Number 10-30 & 44-64, daily; Number 31-34, 1 time/wk

	P1(3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2(3 times/wk)	P2 (Daily)
Control	6.0	6.7	6.3	7.6	8.3
TS	4.2**	5.2**	5.9	5.9**	7.0**

**Means differ, $P < .01$. **Means differ, $P < .05$.

Figure 5. Percentage of Abnormal Sperm Cells

Table 1. Comparison of excised testicular characteristics of control (C) and large testes size line (TS) boars

Characteristics	C	TS
No. of boars	18	23
Average slaughter wt., kg	169.8 ± 3.1	173.8 ± 3.2
Trimmed testes wt., g	286.5 ± 13.7	359.5 ± 11.2**
Epididymal wt., g	60.8 ± 2.5	79.2 ± 2.1**
Tunica wt., g	27.9 ± 2.0	36.2 ± 1.6**
Parenchymal wt., g	255.1 ± 12.3	318.0 ± 10.1**
Total testicular sperm, billion	39.0 ± 2.9	57.7 ± 2.4**
Daily sperm production, billion	8.9 ± 0.7	13.2 ± 0.5**
Total sperm reserves, billion	101.6 ± 6.6	138.9 ± 5.4**

**Means for control and TS differ, $P < .01$.

(Figure 5). The lines responded similarly over collection number and at each period except the rest period; TS boars had a lower ($P < .01$) percentage of abnormal sperm cells than C boars. During both treatments, the frequency of abnormalities was greater ($P < .01$) for boars at the older age (5.1 vs 6.8% at 3 collections per week, and 6.0 vs 7.6% at daily collections).

Concentration of sperm cells (Figure 6) and total number of sperm cells per ejaculate (Figure 7) were greater ($P < .01$) for TS boars at each period. Concentration declined linearly during both periods of three collections per week, and then declined quadratically during both periods of daily collections. The response in total sperm cells was similar.

Although TS boars had less volume of semen than C boars (Figure 3), because sperm concentration of the semen was greater (281.8 vs 246.8 million cells per mL at 3 collections per week, and 156.3 vs 126.7 million cells per mL at daily collections), total number of sperm cells per ejaculate was greater for TS boars. The advantage for TS boars was 5.9 billion cells at three collections per week, and 4.3 billion cells at daily collections. Older boars had greater concentrations of sperm cells in the semen and larger numbers of sperm cells per ejaculate ($P < .01$) than younger boars at both three collections per week and daily collections.

Means for characteristics of excised testes for TS and C boars are in Table 1. Body weights were similar for boars of the two lines, but for all other traits, means were greater ($P < .01$) for TS boars than C boars. TS boars had larger testes and epididymides than C boars and produced more sperm cells per day and had greater numbers of sperm in the epididymides.

Discussion

At the end of 10 generations the difference in predicted weight of paired testes at 150 d was 218.3 g. In the present experiment, the difference between TS and C boars in weight of the right testis was 73 g at approximately 13 months of age, and the difference in



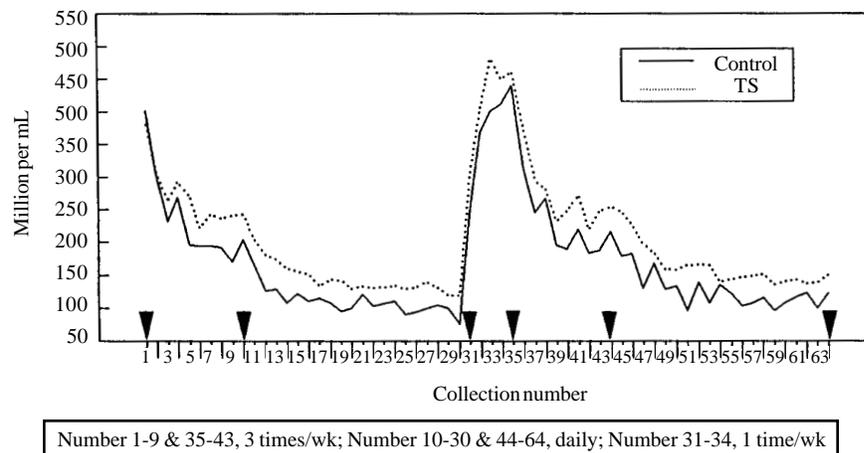
weight of the epididymis was 18.4 g. If these differences are summed and doubled, the difference in weight of paired testes and epididymides was 182.8 g. This result agrees with findings of other experiments that differences in weight of testes between lines was greatest at ages from 130 to 160 d, but that line TS maintains a significant advantage compared to line C to older ages.

The effects of genetic increase in size of testes were that boars produced more sperm cells per day within seminiferous epithelium of the testes and had greater numbers of mature sperm cells in the epididymides. TS boars produced less volume of semen than C boars, perhaps because they took less time to complete the ejaculation. Fluids from accessory sex glands make up a large part of the volume of semen, but the effect of selection for large testes on size and output of these accessory glands was not evaluated.

Because TS boars produced more sperm cells per day and had more sperm cells stored in the epididymis than C boars, concentration of sperm cells in their semen was greater and each ejaculate averaged from 3 to 6 billion more sperm cells. This would provide one to two more doses of semen for artificial insemination from each ejaculate of TS boars than C boars.

Semen for artificial insemination is often diluted to contain 3 to 4 billion sperm cells per dose. The average number of doses per ejaculate when boars were collected three times per week would be 14.4 and 12.7 doses of 3 billion cells for TS and C boars, respectively, at the younger age, and 15.5 and 13.9 doses at the older age. The number of doses would have been 6.6 and 5.4 for TS and C boars at the younger age, and 7.9 and 6 doses, respectively, at the older age when boars were collected daily.

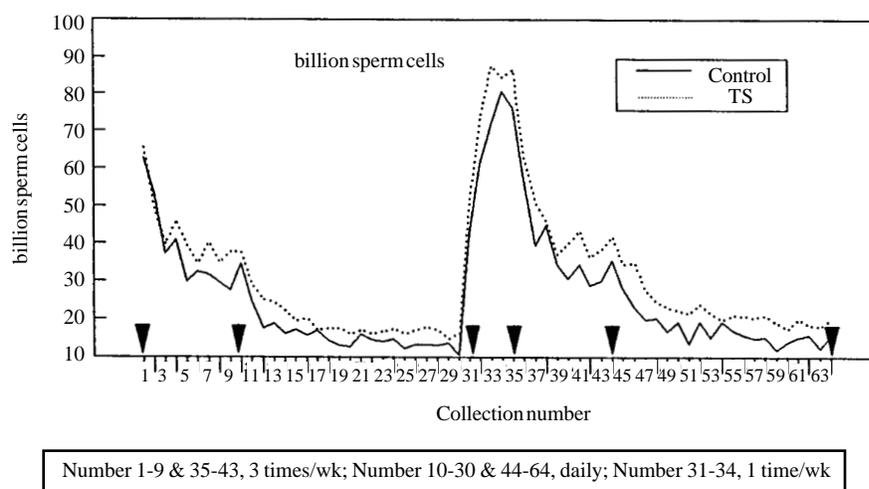
Although percentage motility and frequency of abnormalities are not highly correlated with fertilizing capacity of semen, they are general indicators of quality. Because motility of sperm cells was greater and the frequency of abnormal cells was less for



	P1 (3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2 (3 times/wk)	P2 (Daily)
Control	236.1	118.5	355.4	259.0	135.1
TS	274.2**	150.0**	409.7**	297.3**	169.1**

**Means differ, P<.01. **Means differ, P<.05.

Figure 6. Concentration of Sperm Cells



	P1 (3 times/wk)	P1 (Daily)	Rest (1 time/wk)	P2 (3 times/wk)	P2 (Daily)
Control	38.1	16.3	64.0	41.8	18.0
TS	43.1**	19.8**	74.2**	49.6**	23.8**

**Means differ, P<.01. **Means differ, P<.05.

Figure 7. Total Sperm Cells per Collection

TS boars, TS boars produced semen with more sperm cells without a loss in general quality of these cells.

Testis size is relatively easy to measure with a calipers. Selection for it could be practiced in seedstock herds. We conclude that this selection would increase the number of sperm cells in

each ejaculate of semen. This would be a practical way to increase the efficiency of artificial insemination in swine.

¹Ying-Tsorn is a graduate student, and Rodger Johnson is a Professor in the Animal Science Department, University of Nebraska, Lincoln.