

Spring 2013

Effects of sow, boar, and semen traits on sow reproduction

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Effects of sow, boar, and semen traits on sow reproduction

by

Sungwon Park

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Rodger K. Johnson

Lincoln, Nebraska

May, 2013

Effects of sow, boar, and semen traits on sow reproduction

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University of Nebraska, 2013

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The objective was to estimate the effect of traits recorded in females and in boars and their semen on farrowing rate (FR), total number (TB) and number of stillborn pigs (SB) at birth. Results of 20,569 inseminations in 4,468 sows on 4 farms with semen from 856 boars in 2 AI centers were analyzed. Records on sows included farm, dam line parity and breeding interval (Brdint). Records on boars included number of days rest between collections, and 26 characteristics (e.g., volume, sperm concentration, motility, abnormal heads and tails plus 16 traits that described velocity and path of sperm cell movement). At first time, we were trying to use whole boar semen traits for our analysis; however the attempt was not competitive enough to reveal which semen characteristics had been far more deeply involved in FR, TB and SB. Thus, we used STEPWISE, MAXR and R-square were used for choosing statistically best semen characteristics. Data were analyzed with SAS PROC MIXED in models accounting for fixed effects of farm dam line of sow (Dline) and parity, random effects of sow and boar, and regressions of sow reproductive traits on sow, boar, and semen traits. Models were first fitted with only linear regressions; if important ($P < 0.10$), 2nd models including quadratic effects were fitted. Parity and the interval from 1st insemination (1st estrous during breeding period in gilts, and 1st post-weaning

estrus in sows) to the insemination that resulted in a litter affected ($P < 0.01$) FR, and SB ($P < 0.1$); parity also affected FR, TB and SB ($P < 0.01$). Average FR declined in a quadratic manner by 0.15 as the interval from 1st insemination to insemination of conception increased from 0 to 65 days. Sow reproductive traits were not affected ($P > 0.10$) by number of days between collections (all boars had at least 3 d rest) or sperm concentration. Ten traits (Tmot, Vol, Proximal, Distal, Compos, Head, Tail, VAP, DSL and AOC) describing semen traits affected sow reproduction ($P < 0.10$), but differences across the range of variation were relatively small.

Key Words: Boar, Semen, Sow Reproduction

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Chapter 1: Introduction

In swine industry, in these days, Yorkshire (Y), Landrace (L) and F1 (Y*L) are widely used to produce more piglets as maternal lines because of their milking ability, mothering ability, and fecundity. Berkshire, Hampshire, Duroc, Pietrain, and composites among these and other breeds are widely used to improve the quality of meat and efficiency of growth as sire lines. They have less number of piglets than maternal lines, but their meat quality (Berkshire) is better or they have superior lean growth rates (Duroc, Hampshire, and composites). Most previous research of farrowing rates, total born, number of born alive, etc., has focused on females and the results show that these traits are highly correlated with maternal lines' characteristics. With natural service, male (boar) effects on these traits were sometimes significant, but usually explained less than 5% of the total variation (often 2 to 3%) and were not considered important.

There is minimal natural service occurring in today's swine industry. Artificial insemination (AI) is used extensively by today's swine producers as procedures of semen collection, preservation, and transportation from AI centers to swine farms have improved greatly in the last 20 years, making AI a much more efficient process in terms of time and labor and in efficiency of boar use than natural service.

During the earlier years of AI, semen quality was measured primarily by volume and by traits that could be observed microscopically such as sperm concentration, motility, and certain abnormalities of the head and tail. Advances in digital technology have led to development of instruments that measure additional semen quality traits. Minitube's Sperm Vision® CASA System (MINITUBE GmbH, Hauptstrasse 41, 84184 Tiefenbach,

Germany) is such a system. In addition to the more commonly recorded semen traits, it also records a number of characteristics describing rate and direction of motion and traits describing the morphology of the sperm cells. The objective of this research was to estimate the effects of these semen traits and additional traits recorded in females and in boars on farrowing rate (FR), total number (TB) and number of stillborn pigs (SB) at birth.

The presupposition of sperm fertilizing power can have a great economic effect for breeding herds when AI is used (Gadea, 2005). Understanding the correlation of specific motion characteristics of individual semen collections can improve the efficiency of boar semen production (Didion BA, 2008). In addition, if we can predict the number of total born and stillborn in AI semen stage at the same time, we can amplify the effect. Thus, we use the whole semen traits to find out the meaningful values for the predictions.

Chapter 2: Literature Review

In mammals, normal zygotes are formed from healthy spermatozoa and oocytes and the zygotes pass through several differentiation processes in order to generate as individuals. The production of healthy oocytes and the conditions of the uterus for implantation are female characteristics. The production of healthy spermatozoa is a male characteristic.

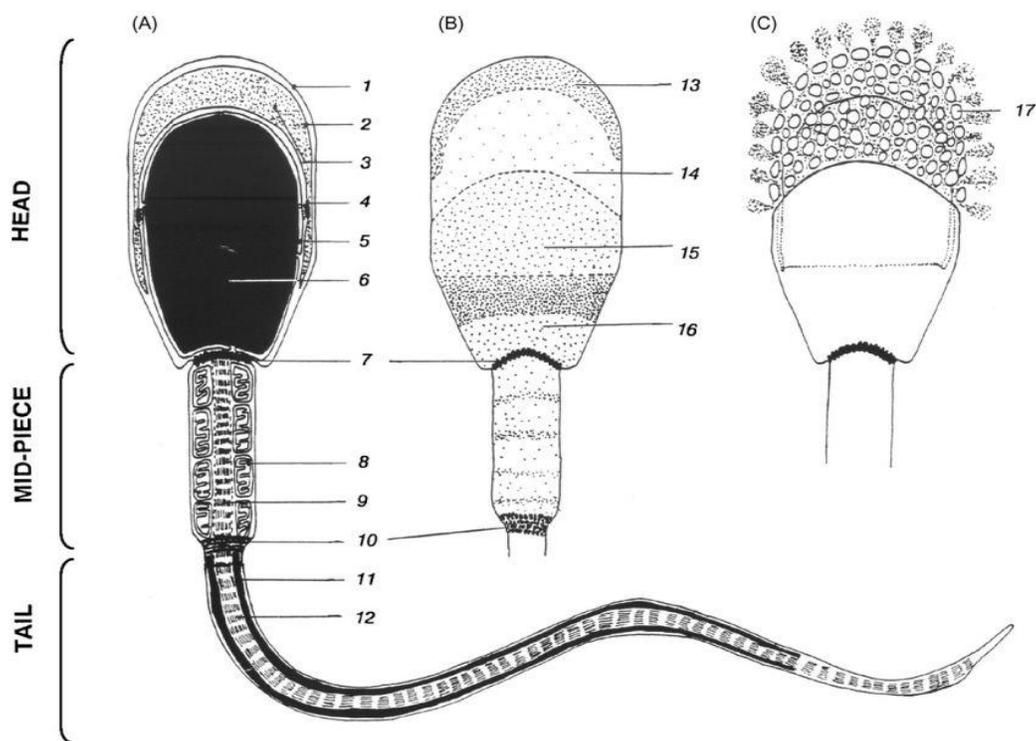
Composition of Sperm

Figure 1. illustrates the general morphology of the sperm cell. The main components are the head, which contains the DNA that is transmitted to progeny, a midpiece that is involved in energy production for the tail piece, and the tail that allows the sperm to move (swim).

Sizes of spermatozoa from some livestock animals and human (data from Cummins and Woodall, 1985) are in Table 1. Although length of individual parts of the sperm cell varies somewhat across species, total length does not vary greatly.

The sperm flagellum is long and thin in most animal species. For motility, the flagellum use ATP that is generated from mitochondria in the midpiece of sperm. In all animal species, the color of sperm is creamy white and the temperature is approximately 37.5°C.

Figure 1. Composition of boar sperm cell (Boerke et al., 2008).



(Panel A) A sectional view of the sperm cell. 1, plasma membrane; 2, outer acrosomal membrane; 3, acrosomal enzyme matrix; 4, inner acrosomal membrane; 5, nuclear envelope; 6, nucleus; 7, posterior ring and neck; 8, mitochondria; 9, proximal part of the flagellum; 10, annular ring; 11, fibrous sheath; 12, axoneme + outer dense fibers. (Panel B) A surface view of the sperm head and mid-piece with the subdomains. 13, apical ridge; 14, pre-equatorial; 15, equatorial; 16, post-equatorial. (Panel C) The acrosome reaction. 17, the mixed vesicles formed during the acrosome reaction via multiple fusions between the plasma membrane and the outer acrosomal membrane.

Table 1. Length of individual parts of the sperm cell^a

Species	Length (μm)			
	Head	Midpiece	Tail	Total
Bull	6.8	9.8	36.9	53.5
Boar	8.5	10.0	36.1	54.6
Ram	8.2	14.0	43.0	65.2
Horse	7.0	9.8	43.8	60.6
Human	4.5	4.0	48.0	56.5

^aCummins and Woodall, 1985

Swine artificial insemination (AI)

Swine artificial insemination (AI) was first performed by Ivanow in Russia in the early 20th century (Ivanow, 1907) and the AI procedure was established at Russian state farms in the 1930s (Rodin and Lipatov, 1935; Milovanow, 1938). The success of AI procedures is highly correlated with quality of semen, adroitness of staff in estrus detection and staff competence of insemination (Holt et al., 1997). AI is used extensively in order to break down the inefficiency of natural mating in the swine industry. It contributes to the efficient distribution of marvelous genetic resources and aids in efficiently managing a genetic program. AI centers are always trying to minimize the variation of their semen quality (Broekhuijse et al., 2012).

Semen collection methods

There are three major semen collection methods for boars which are artificial vagina method, gloved hand and electroejaculation methods. The artificial vagina method was first attempted (Holst SJ., 1945) to collect boar semen, but it is underused now, because the gloved hand method is so easy and efficient. In the gloved hand method, vinyl gloves are preferred than latex gloves because some latex gloves could contain spermicidal materials (Ko JCH et al., 1989). The electroejaculation method is specifically used for collecting difficult/dangerous-to-handle boars. Anesthesia is needed for electroejaculation, thus the method is not often use in the field because of the risk and added costs.

Semen examination

Sperm examination is an essential progress to anticipate fertility rate, the number of total born and the number of stillborn of AI. From the examination, using the microscope, several traits of semen were already investigated and measured by the sperm quality. The traditional microscopic semen traits are the number of spermatozoa, semen volume (Vol), sperm concentration (Con), sperm motility (Mot), and normality of external appearances of the sperm. The number of spermatozoa has important role for fertilization and it has certain threshold values (Saacke et al, 1994). The number of spermatozoa in an ejaculate has variation between pig breeds (Kommissrud et al, 2002), and also ejaculate semen Vol has variation (Kondracki, 2003). Alm et al. (2006) reported a general threshold number of spermatozoa for higher fertility rate ($84.3\% \pm 3.4$) in an AI semen dose was 3×10^9 spermatozoa when boar semen had good quality (Boars with $<70\%$ of normal spermatozoa had been excluded). Con is an indicator for the number of sperm (Shiple, 1999). Con can be measured by visual evaluation. The evaluation for Con by the semen color had three categories which were a watery to opalescent semen sample ($0 \sim 200 \times 10^6$ per mL), a milky semen sample ($200 \sim 500 \times 10^6$ per mL) and a creamy semen sample ($500 \sim 1000 \times 10^6$ per mL). These Con categories are very subjective, thus it is not good to adapt to the AI center (Vyt P, 2007). Con also can be measured by using a hemacytometer or photometric means. Photometric means method uses light transmission absorbance to calculate Con (Shiple, 1999).

In vivo, according to S. Tardii et al. (1999), Mot was the important trait for quality estimate of spermatozoa. Sperm Mot could be easily evaluated after semen collection. Ejaculated sperm Mot is an important condition of semen evaluation, and as time goes on

it is decreased so it should be tested as soon as possible after ejaculation (Rozeboom KJ, 2000). Most AI studs have a normal motility cutoff level of 70% or greater for use of semen for insemination. The Mot is measured as the percentage of sperm that can normally move forward. Outside factors, such as heat, cold, any residual substance of semen collection equipment, and pH or osmolality of the extender can produce irreversible affect to the semen motility. Seminal plasma is important for motility. Mixing of sperm and seminal plasma causes pH and bicarbonate concentration increase and spermatozoa get motility from these factors (Rodriguez-Martinez et al., 1990). Interestingly, the relationship between Mot and fertility is controversial because of the difference on experimental condition; however, mean Mot is a good parameter in the seminal analysis and eliminates low quality semen (Gadea et al., 1998).

Classical semen evaluation methods, such as sperm volume, concentration, progressive motility, percent of viable cell and acrosome morphology, provide a poor prediction for farrowing rate and litter size, because these methods only can detect very poor semen quality (Gadea, 2005).

The development of digital equipment allows additional semen and more detailed sperm characteristics to be recorded. In addition to the traditional semen traits, morphological characteristics of the sperm head and tail, and traits that describe rate and direction of motion can be recorded. These traits may be combined in a composite score intended to describe overall semen quality.

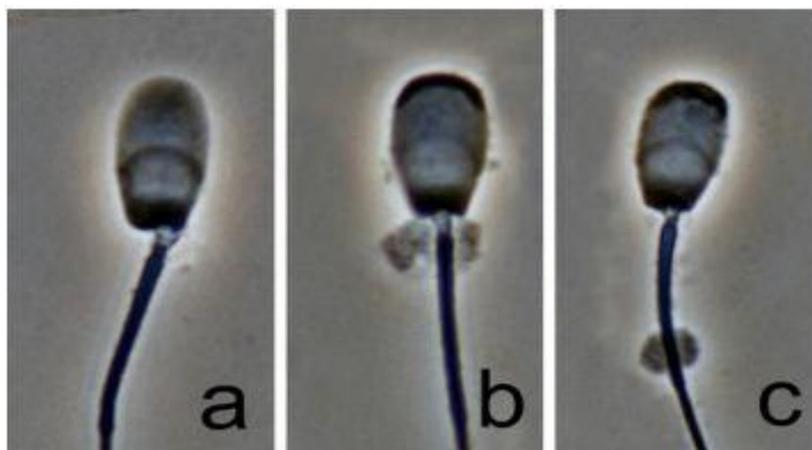
In addition to motility, morphological characteristics of the sperm have been reported to affect fertility. According to the Hirai et al. (2001), sows inseminated with sperm with

elongated heads had low non-return rates, defined as percentage of sows not appointed for a second insemination within a period of minimum 60 to maximum 90 days after the first insemination. However, sows inseminated with elongated head sperm had smaller litters (less than 10 piglets per litter). They also found significant correlations ($P < .01$) between non-return rate and sperm cell length ($r = -0.85$) and width to length ratio ($r = .87$). However, in that research sperm were classified simply as percentage with normal appearing heads and tails.

In seminiferous tubules, whole sperm have cytoplasmic droplets and the droplets are the remains of cytoplasmic linkage between several sperm during spermatogenesis. Most droplets are shed when they are mixed with semen elements at ejaculation (Morgan Morrow, Swine News July, 1998 • Volume 21, Number 6). Gary C. Althouse (Swine Health and Production 1998 6:128) mentioned, “Using ejaculates for AI, semen have fewer than 20% morphologically abnormal sperm, with no more than 15% attributable to cytoplasmic droplets.” According to Waberski et al., (1994a) sperm which had high percentage of distal and proximal cytoplasmic droplets had a negative correlation to both percentage pregnant and litter size. Proximal droplets were defined as cytoplasmic droplets at the neck/upper mid-piece region and Distal droplets were defined as cytoplasmic droplets at the terminal portion of the mid-piece. Rozeboom (2000) mentioned that the increasing percentages of proximal cytoplasmic droplets gradually decreases farrowing rate and litter size. Distal cytoplasmic droplets are more commonly found than proximal cytoplasmic droplets. Although there are limited scientific references regarding the impact of cytoplasmic droplets in boar ejaculates, it has been suggested that the incidence of plasmas droplets should not exceed 15% when semen is

stored for extended periods of time, at least 2 days (Rozeboom, 2000). Generally, Distal-cytoplasmic droplets are considered a less serious condition than proximal cytoplasmic droplets.

Figure 2. Existence and non-existence of cytoplasmic droplet of boar^a.



(a)absence of cytoplasmic droplet, (b) Proximal cytoplasmic droplet, (c) Distal cytoplasmic droplet

^aLopez-Fernandez et al., 2008

Recent red deer fertility trials (Gomendio et al., 2006b) show that sperm swimming velocity and ratio of normal sperm are related to fertility when sperm numbers are kept constant. ALH defined as the maximum of the measured width of the head oscillation as the sperm cells swam (Broekhuijse et al., 2012).

Chapter 3: Materials and Methods

Animals

Records were provided by Danbred NA. All sows and boars records collected from January 2007 until December 2009 were analyzed. Data are from four nucleus sow farms and two artificial insemination (AI) facilities and included characteristics of semen from 856 boars used for 20,569 inseminations in 4,468 sows. All performance records were obtained from materials gained from the web-based database used by Danbred NA.

General management and collection of data

Nucleus farms contained pure lines of Danbred's maternal lines and their terminal sire line. Two farms contained sows of a maternal line that originated from the Danish Landrace breed. The other two farms contained sows of Danbred's other maternal line that originated from the Yorkshire breed and their terminal sire line that originated from the Duroc breed. The farms and the breeds are designated as Adams (Landrace), Brainard (Duroc and Yorkshire), Fairbury (Landrace), and Oneida (Duroc and Yorkshire).

All replacement gilts on the farms were performance tested either on the farm of origin or at Danbred's central performance test center and were selected based on Danbred's maternal index (Landrace and Yorkshire) or terminal sire index (Duroc). They were moved to the breeding area shortly after completing the performance test and after an adjustment period were inseminated with semen from boars of the same breed. They were inseminated daily while in estrus, monitored for signs of return to estrus and repeatedly at additional estrous cycles until diagnosed pregnant or culled.

Litters were weaned at approximately 20 d of age. Sows were observed for symptoms of estrus and mated each day while in estrus and repeatedly at additional estrous cycles until diagnosed pregnant or culled. Most sows were allowed no more than four litters, however, a small number of sows had as many as seven litters before being culled. Traits recorded in females included parity, year at insemination, month at insemination, the number of inseminations during the cycle, farrowing year, farrowing month, whether the insemination resulted in a pregnancy (0 = not pregnant, 1 = pregnant), and number of total born, stillborn and live pigs at day 5 (LP5) in the litter they produced. From these data a trait named breeding interval was calculated (the number of days from 1st opportunity to be inseminated to the insemination that resulted in a litter or the last insemination before culling, range from 0 to 154. Gestation length was calculated as the difference between the farrowing date and mating (service) date. Age at insemination was also calculated from the difference between service date and birth date of sows and gilts. Table 2 contains a description of sows on each farm.

Table 2. Description of pure breed females^a.

Farm	Breed	Parity	Number of Females	FR (%)	TB	SB	Age at Insemination, days		
							Range	Mean	SD
Adams	LL	1	1590	87.5	11.7	1.8	185-411	234.0	31.1
		2	1245	75.7	12.2	1.7	334-575	388.1	39.2
		3	876	71.1	12.1	2.2	481-716	543.5	39.6
		4	395	69.1	11.5	2.4	620-849	698.9	44.3
		>4	105	52.4	10.1	3.1	790-1003	885.7	52.5
Brainard	YY	1	629	91.6	11.2	1.1	204-362	251.0	21.7
		2	461	70.5	12.2	0.9	349-519	400.6	26.5
		3	311	77.5	12.8	1.3	502-623	548.1	29.6
		4	194	85.1	12.3	1.4	643-790	695.9	30.6
		>4	148	76.4	12.0	1.7	783-1011	842.4	49.4
	DD	1	1210	88.5	8.2	1.2	172-440	253.3	30.5
		2	741	80.3	9.4	1.3	311-562	409.7	34.9
		3	449	77.3	9.5	1.6	357-718	554.9	37.2
		4	207	73.4	9.3	1.6	619-804	698.3	33.3
		>4	57	75.4	8.1	2.3	783-908	842.0	32.5
Fairbury	LL	1	1276	89.0	11.6	2.0	186-403	240.9	29.7
		2	1405	70.9	12.1	2.1	332-846	453.6	119.5
		3	714	70.6	12.0	2.4	369-724	549.1	34.4
		4	93	67.7	11.4	2.6	654-777	702.8	24.8
		>4	117	75.2	11.2	3.4	799-1094	865.3	65.1
Oneida	YY	1	1849	83.6	10.8	1.1	190-448	243.3	29.6
		2	1334	75.6	12.0	0.8	329-563	391.3	33.0
		3	761	72.1	12.1	0.9	474-724	540.4	32.3
		4	352	64.2	11.7	1.0	615-769	679.7	29.0
		>4	163	63.8	11.3	1.5	762-882	829.5	32.4
	DD	1	1393	81.5	7.9	1.1	182-410	237.9	27.6
		2	1014	72.9	8.8	1.1	307-571	390.3	35.2
		3	578	75.1	9.3	1.2	466-716	542.4	36.5
		4	279	69.9	8.8	1.5	616-804	687.0	32.2
		>4	91	74.7	8.5	1.8	760-977	835.5	45.8

^aCrossbreed sows data were eliminated.

Boar management and Sperm traits

Boars were pureline boars of the maternal Landrace and Yorkshire lines and of the Duroc terminal sire line. After completing the performance test at Danbred's central test facility, they were transported to one of two AI centers where semen was collected and distributed to sow farms. Standard feeding and management practices were used.

Boar age at time of semen collection, number of days since the last collection, and whether semen was collected during the morning (AM) or afternoon (PM) work hours were recorded. The management practice used was to rest boars at least three days between collections. Actual number of rest days was calculated from the data. Volume of semen was recorded at the time of insemination. The raw ejaculated boar semen was diluted with extender at a 20:1 ratio. A diluted sample was then placed in a leja slide chamber. SpermVision® CASA System evaluates the different types of movement which indicate motility across 7 fields within the chamber. This takes roughly 20 seconds and reports analysis by individual cell, per field, and sample (average of all fields). Collected characteristics of the semen and sperm cell traits are:

1. Semen volume (Vol): Total volume of the raw ejaculate expressed in milliliters (mL).
2. Sperm Concentration (Con): Number of spermatozoa per ml expressed in billions (10^9).
3. Total Motility (Tmot): the percentage of spermatozoa that had any movement of the sperm head.

4. Progressive Motility (Pmot): the percentage of spermatozoa which moved in a forward direction.
5. Low Motility (Lmot): the percentage of spermatozoa that are alive, but move very little in the forward direction.
6. Head: the percentage of normal head.
7. Tail: the percentage of normal tail.
8. Proximal: the percentage of cells that had no cytoplasmic droplets on proximal area.
9. Distal: the percentage of cells that had no cytoplasmic droplets on distal area.
10. Composite score (Compos): The product of % Motile multiplied by % Normal (Normal determined by normal morphology. Sperm have no abnormal head and tail, and cytoplasmic droplets) multiplied by % Viable (Viable is determined by multiplying Total Cells (live and dead) by % Motile by % Normal).
11. Distance Curved Line (DCL): the actual distance (microns) that sperm cell traveled from the beginning to the end of the analysis period.
12. Distance Average Path (DAP): the distance (microns) of the average path of the sperm cell from the beginning to the end of the analysis period.
13. Distance Straight Line (DSL): the distance (microns) that the sperm traveled in a straight line from the first frame to the last frame of the analysis.
14. Velocity Curved line (VCL): the speed that the sperm cell traveled across the curved line from the beginning to the end of the analysis period measured in microns per second.

15. Velocity Average Path (VAP): the speed that the sperm cell traveled across the average path from the beginning to the end of the analysis period measured in microns per second.
16. Velocity Average Path (VSL): the speed that the sperm cell traveled in a straight line from the beginning to the end of the analysis period measured in microns per second.
17. Amplitude of lateral head displacement (ALH): the magnitude of lateral displacement of a sperm head about its average path (microns). It is expressed as maximum displacement.
18. Beat cross frequency (BCF): the speed measured in Hertz that the head of the sperm cell is moving from side to side during the measurement period.
19. Straightness (STR, VSL/VAP): the relationship between the velocity of the straight line and the velocity of the average path during the measurement period.
20. Wobble (WOB, VAP/VCL): the relationship between the velocity average path and the velocity curved line during the measurement period.
21. Linearity (LIN, VSL/VCL): the relationship between the velocity of the straight line and the velocity of the curved line during the measurement period.
22. Hyperactive (HYP): the percentage of the ejaculate meeting hyperactive motion criteria.
23. Average orientation change (AOC): the average change in orientation of the head of the sperm cell between frames during the measurement period measured in degrees.

24. Linear motion (Linear): the percentage of the ejaculate meeting linear motion criteria.

25. Non-Linear motion (Nlinear): the percentage of the ejaculate meeting non-linear motion criteria.

26. Curvilinear motion (Curv): the percentage of the ejaculate meeting curvilinear motion criteria.

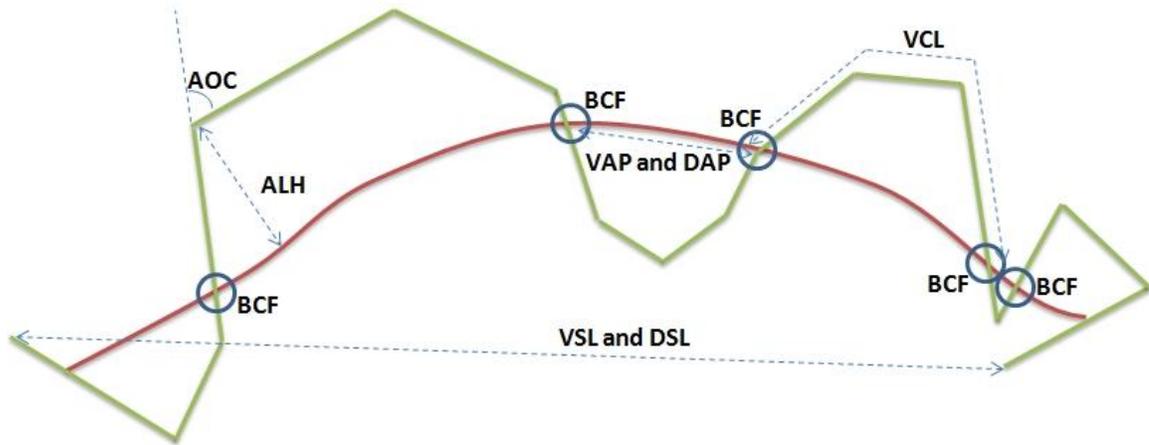
The mean and standard deviation of semen traits are in Table 3. Figure 3 illustrates sperm movement measurements as recorded by the CASA system.

Table 3. Mean and standard deviation (SD) of semen traits^a

Parameter	Value	SD	Parameter	Value	SD
Vol (ml)	193.89	87.29	VCL (microns/sec)	117.25	41.07
Con (10 ⁹ /ml)	0.50	0.24	VAP (microns/sec)	62.99	17.07
Tmot (%)	87.51	7.12	VSL (microns/sec)	40.46	20.50
Pmot (%)	78.78	10.95	ALH (microns)	8.20	9.15
Lmot (%)	8.72	5.90	BCF (hertz)	25.15	11.42
Head (%)	99.53	1.02	STR (VSL/VAP)	0.69	0.09
Tail (%)	98.96	1.67	WOB (VAP/VCL)	0.50	0.06
Proximal (%)	99.30	1.40	LIN (VSL/VCL)	3.30	6.90
Distal (%)	97.82	2.20	AOC (degree)	19.27	7.07
Composite score	84.71	7.49	Hyp (%)	12.12	9.76
DCL (microns)	57.10	15.63	Linear (%)	12.96	10.16
DAP (microns)	29.34	7.09	Nlinear (%)	30.31	13.49
DSL (microns)	21.73	5.83	Curv (%)	9.27	4.93

^a Measured by The Sperm Vision®, SD=standard deviation.

Figure 3. Standard terminology for variables measured by CASA systems^a



^aModified from WHO(2010)

Statistical Analyses

Model Building: Application of STEPWISE, MAXR, and R-Square Procedures

Sow farrowing rate and numbers of total born and stillborn pigs per litter are characteristics of the sow that are affected by farm, management, and by both sow and semen characteristics. Some effects can be considered fixed, such as farm, dam line of sow (Dline), and parity of the sow. Other effects, such as age of boar when semen was collected and characteristics of the semen can best be modeled with regression, and other effects such as permanent characteristics of sows and boars are best modeled as random effects.

Because of the large number of variables recorded in semen and sows, the first step was to eliminate from further consideration variables recorded in semen that did not affect sow reproduction. Farrowing rate is a binomial trait and should be analyzed with a generalized linear model, whereas number of pigs per litter can be considered a normally

distributed trait. In both cases, a mixed model with fixed and random effects including regression variables is appropriate.

Because of the large dataset and the large number of regression variables, it was not practical to use mixed model methods to arrive at a final model for each trait. Therefore, the SAS procedures of **STEPWISE**, **MAXR**, and **R-Square** were used to identify which semen characteristics to include in final models. They were applied to the overall dataset without fitting fixed and random effects as these procedures only fit regression variables and do not allow fitting fixed and random effects. When compared with a more correct model, these procedures also underestimate standard errors of regression coefficients and produce P-values that are too small. They were used only as a first step in eliminating unimportant variables from additional consideration. Variables determined to be important by these methods, as described below, were then included in final mixed models to estimate regression coefficients.

As mentioned before the objective was to determine whether characteristics of the semen and certain characteristics of the sow affected sow reproduction. Three regression methods, STEPWISE, MAXR, and R-Square, as described in Cite, were first used to eliminate characteristics that did not affect ($P > 0.10$) sow reproduction. Characteristics of the sows and all variables recorded in boars were considered. These included Vol, Con, motility (Tmot, Pmot, Lmot), Head, Tail, position of cytoplasmic droplets (Proximal and Distal), Compos, distance (DCL, DAP and DSL), velocity (VCL, VAP, VSL, WOB [VAP/VCL], LIN [VSL/VCL] and STR [VSL/VAP]), ALH, BCF, AOC, HYP, and motion (Linear, Nlinear, Curv).

In the STEPWISE method variables are added one by one to the model, and the F statistic for a variable to be added must be significant at the $SLENTRY=$ level, which was set at 0.10. After a variable is added, however, the method looks at all the variables already included in the model and deletes any variable that does not produce a significant F statistic. Another variable is added to the model only after this check is made and the necessary deletions are accomplished can another variable be added to the model. The STEPWISE process ends when none of the variables outside the model has an F statistic significant at the $SLENTRY=$ level and every variable in the model is significant at the specified level, or when the variable to be added to the model is the one just deleted from it.

The maximum R^2 (MAXR) technique does not settle on a single model. Instead, it tries to find the "best" one-variable model, the "best" two-variable model, and so forth, although it is not guaranteed to find the model with the largest R^2 for each size. The MAXR method begins by finding the one-variable model producing the highest R^2 . Then another variable, the one that yields the greatest increase in R^2 is added. Once the two-variable model is obtained, each of the variables in the model is compared to each variable not in the model. For each comparison, the MAXR method determines if removing one variable and replacing it with the other variable increases R^2 . After comparing all possible switches, the MAXR method makes the switch that produces the largest increase in R^2 . Comparisons begin again, and the process continues until the MAXR method finds that no switch could increase R^2 . Thus, the two-variable model achieved is considered the "best" two-variable model the technique can find. Another variable is then added to the

model, and the comparing-and-switching process is repeated to find the "best" three-variable model, and so forth.

The difference between the STEPWISE method and the MAXR method is that all switches are evaluated before any switch is made in the MAXR method. In the STEPWISE method, the "worst" variable might be removed without considering what adding the "best" remaining variable might accomplish.

The RSQUARE method finds subsets of independent variables that best predict a dependent variable by linear regression. It performs all possible subset regressions and displays the models in decreasing order of R^2 within each subset size. Other statistics are available for comparing subsets of different sizes. The subset models selected by the RSQUARE method are optimal in terms of R^2 for the given sample, but they are not necessarily optimal for the population from which the sample is drawn or for any other sample for which one might want to make predictions. The RSQUARE method is a useful tool for exploratory model building. It differs from the other selection methods in that RSQUARE always identifies the model with the largest R^2 for each number of variables considered. The other selection methods are not guaranteed to find the model with the largest R^2 .

Farrowing rate is a binomial trait and should be analyzed with a generalized linear model as can be done with PROC GLINMMIX in SAS. Such an analysis was attempted but would not solve due to memory constraints – the data set was too large. Therefore, all traits were analyzed with the PROC MIXED method of SAS.

Models for each trait included farm, parity and dam line of sow as fixed effects, service year, service month, sow, boar, and the error term as random effects, and regression variables identified by the three-regression procedures described above. Breeding interval of sows (Brdint) was significant for all traits and the linear and quadratic effect was included in all models. Data were analyzed with the SAS PROC MIXED procedure (SAS Inst. Inc., Cary, NC). In models accounting for fixed effects of breed and parity of sow, random effects of sow and boar, and regressions of sow reproductive traits on sow, boar, and semen traits.

Models were first fitted with only linear regressions. The general model was:

$$Y = \mu + \text{Farm} + \text{Dline} + \text{Parity} + \text{Sery} + \text{Serm} + \text{Sow} + \text{Boar} + \text{Brdint} + \text{Brdint}^2 + \text{Boarage} + \text{Drest} + \sum X_i + \text{error } (\varepsilon),$$

Y was farrowing rate, total born and stillborn,

μ was the overall trait mean, Farm, Dline, Parity, are fixed effects, Syear, Serm, sow and boar are random effects, Brdint, Boarage, and Drest are regression variables, and X_i represents characteristics of semen fitted as regression variables.

Some semen traits identified by the three-regression models that were included in these models were not significant ($P > 0.10$) when included in the mixed model procedure. Further model refinement was accomplished by removing those X variables from the model and including the quadratic effect of those X variables that were significant. Least squares means at specified values of each X variable, holding other variables fixed at the mean value, were generated in the final analysis and plotted to illustrate responses. When the quadratic effect was not significant,

The final model for each trait was: only the linear coefficient was included in producing least squares means. The final model for each trait was:

$$Y (\text{FR}) = \mu + \text{Farm} + \text{Dline} + \text{Parity} + \text{Sery} + \text{Serm} + \text{Brdint} + \text{Brdint}^2 + \text{Sow} + \text{Boar} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{DSL} + \text{VAP} + \text{STR} + \text{WOB} + \text{Vol}^2 + \text{DSL}^2 + \text{VAP}^2 + \text{STR}^2 + \text{WOB}^2 + \text{error } (\epsilon)e,$$

$$Y (\text{TB}) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Parity} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Vol}^2 + \text{error } (\epsilon), \text{ and}$$

$$Y (\text{SB}) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Parity} + \text{Boarage} + \text{Drest} + \text{Head} + \text{Tail} + \text{Proximal} + \text{Distal} + \text{Compos} + \text{VCL} + \text{VAP} + \text{STR} + \text{WOB} + \text{Head}^2 + \text{Tail}^2 + \text{Proximal}^2 + \text{Distal}^2 + \text{Compos}^2 + \text{VCL}^2 + \text{VAP}^2 + \text{STR}^2 + \text{WOB}^2 + \text{error } (\epsilon).$$

Chapter 4: Results

Estimates of variance components from the final model for each trait are in Table 4. .

Table 4. Variance components for Linear model.

	FR	Proportion	TB	Proportion	SB	Proportion
Sow	0.00203	1.22%	0.6163	5.46%	0.1013	3.08%
Boar	0.00552	3.33%	0.5485	4.86%	0.05646	1.71%
Sery ^a	0.00095	0.57%	0	0.00%	0.1404	4.26%
Serm ^b	0.00028	0.17%	0.02948	0.26%	0.02017	0.61%
Residual	0.1568	94.70%	10.1021	89.43%	2.9757	90.34%
Total ^c	0.16557		11.2964		3.29403	

Sery^a: service year of sow (2007, 2008, 2009), Serm^b: service month of sow, Total^c: summation of the values of Sow, Boar, Sery, Serm and Residual.

Variance components are estimated for the random effect portion of our model. The percentage of variation due to sows ranged from 1.22% to 5.46%. The percentage of variation due to boars ranged from 1.71% to 4.86%. The percentage of variation for Sery and Serm were quite small: none of the random effects accounted for very much of the total variation. However, the residual values contributed huge variations to the total variation in all parts. Our interests were the variation of sows and boars. Especially, our greatest concern was how much boar variance influenced sow reproduction. However, they provided very small effects. Another interesting thing was that the repeatability of the litter size of sow is generally around 20%, however, according to our data, it was just 5.46%. The variation of sows and boars were pretty small and unexplained variations, residual, which were not associated with sow and boar occupied huge part of the total variance.

Farrowing rate (FR)

In selected semen characteristics analysis, Vol, Tmot, STR, VAP, DCL, Distal and DSL were included in linear mixed models to estimate effects of regression variables for FR.

Linear regression model for FR was,

$$Y (\text{FR}) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Tmot} + \text{STR} + \text{VAP} + \text{DCL} + \text{Distal} + \text{DSL} + \text{error} (\epsilon),$$

Vol, Tmot, VAP, Distal and DSL were significant in the linear regression model ($P < 0.1$), however, STR and DCL were not. Thus, STR and DCL were eliminated for final quadratic regression model. In final model, only Vol showed statistical difference (Table 5). Quadratic regression model for FR was,

$$Y (\text{FR}) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Tmot} + \text{VAP} + \text{Distal} + \text{DSL} + \text{Vol}^2 + \text{Tmot}^2 + \text{VAP}^2 + \text{Distal}^2 + \text{DSL}^2 + \text{error} (\epsilon),$$

Vol only showed significant in the quadratic regression model ($P < 0.1$) and other semen characteristics had non-significant P-value (Table 5).

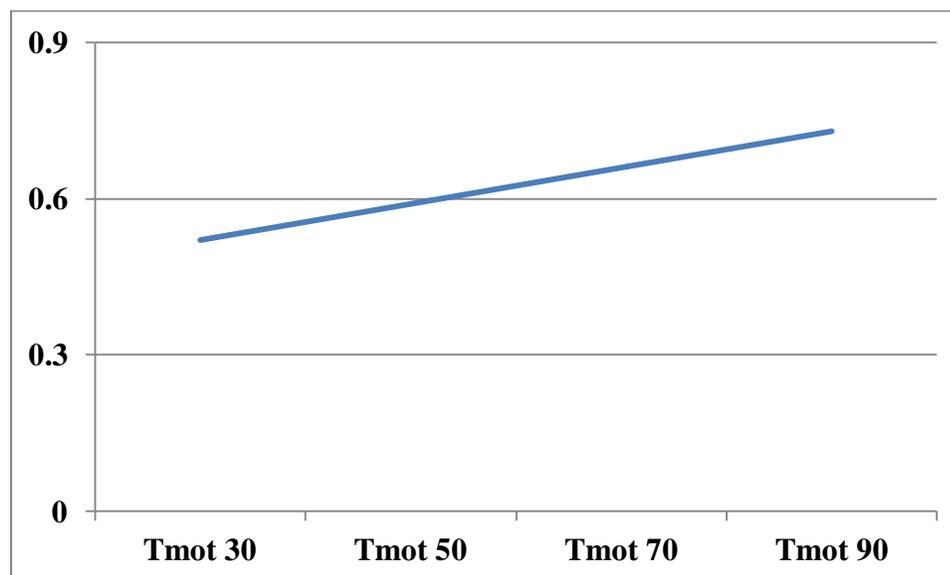
Table 5. Linear and quadratic regressions(b) and standard errors (se) and P-values from final model of farrowing rate (FR).

Linear regressions			Linear and quadratic regressions		
	b±se	P-value		b±se	P-value
Brdint, day	-0.00430±0.000406	<.0001	Brdint, day	-0.00428± 0.000406	<.0001
Brdint2	0.000033±4.745E-6	<.0001	Brdint2	0.000033±4.746E-6	<.0001
Boarage, day	0.000125±0.000042	0.003	Boarage, day	0.000114±0.000042	0.0069
Drest, day	0.000204±0.000961	0.8323	Drest, day	0.000268±0.000961	0.7803
Tmot, %	0.00349±0.000597	<.0001	Tmot, %	-0.00292±0.005129	0.5696
VAP, microns/sec	-0.00245±0.000851	0.004	VAP, microns/sec	-0.00188±0.002014	0.3503
Vol, Mℓ	-0.00011±0.000047	0.0168	Vol, Mℓ	0.000196±0.000149	0.1891
Distal, %	-0.00305±0.001510	0.0432	Distal, %	-0.07424±0.04862	0.1268
DSL, microns	0.003502±0.001671	0.0361	DSL, microns	0.002794±0.005814	0.6308
			Tmot2	0.000040±0.000032	0.2126
			VAP2	-1.06E-6±0.000014	0.9379
			Vol2	-6.04E-7±0	0.0368
			Distal2	0.000372±0.000254	0.1439
			DSL2	0.000015±0.000107	0.8887

Brdint: breeding interval, Boarage: semen collecting date minus boar birth date, Drest: semen collection interval in AI center, Tmot: total motility. VAP: velocity of average path, Vol: raw semen volume, Distal: distal cytoplasmic droplet, DSL: Distance Straight Line(microns)

We affirmed that when Tmot was increased from 30 to 70, FR also rose from 0.521 (about 52.1%) to 0.730 (about 73%). It was clearly anticipated and typical result (Figure 4.1). One interesting thing was that why Pmot (the percentage of spermatozoa which moved in a forward direction) and Lmot (the percentage of spermatozoa that are alive, but move very little in the forward direction) were not statistically significant effects in FR. Before the analysis, we anticipate that Pmot and Lmot would have some effect to FR, however, they also did not show significant result in any sow reproduction performance. The mean value and SD for Tmot were 87.51% and 7.12, respectively (Table3).

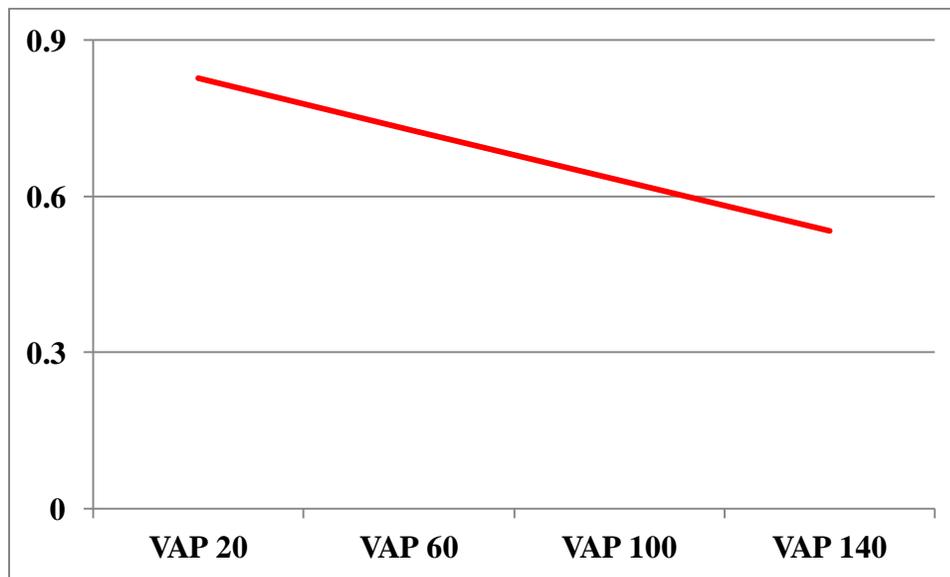
Figure 4.1. Relationship between Tmot and Farrowing rate (FR).



Total Motility (Tmot): the percentage of spermatozoa that had any movement of the sperm head.

FR was decreased from 0.827 (about 82.7%) to 0.532 (about 53.2%), when VAP was increased from 20 to 140 (Figure 4.2). According to Holt et al. (1997), decreasing VAP was associated with higher conception rate. Our VAP result is consistent with Holt's result, however, according to Didion (2008), VAP and DSL had positive correlations with farrowing rate, however the correlation value were pretty small (0.0172 and 0.0147, respectively). Our VAP result is coincide with Holt's result. The mean and SD for VAP were 66.29 microns/sec and 17.07, respectively (Table3).

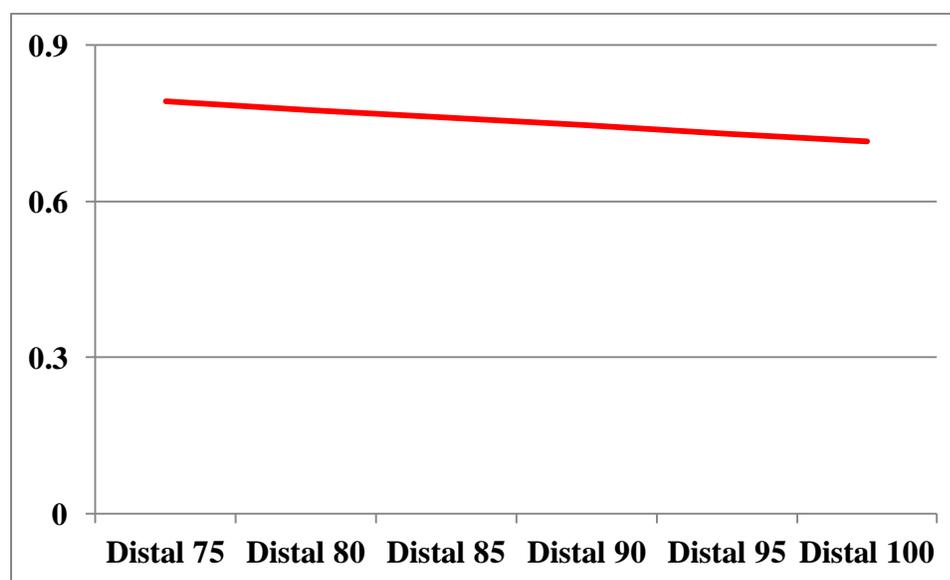
Figure 4.2. Relationship between VAP and Farrowing rate (FR).



Velocity Average Path (VAP): the speed that the sperm cell traveled across the average path from the beginning to the end of the analysis period measured in microns per second.

In sperm morphology, cytoplasmic droplets generally provide undesirable effect to conception rate. From our result, surprisingly, we could not affirm that distal-cytoplasmic droplets provide a negative effect to FR. FR was decreased from 0.791 (about 79.1%) to 0.715 (about 71.4%) when morphologically normal sperms were increased (Figure 4.3). This result was unusual and if this situation was happening repeatedly in AI, we need to rethink about sperm maturation effects for AI semen extender. The mean and SD for Distal were 97.82% and 2.20, respectively (Table3).

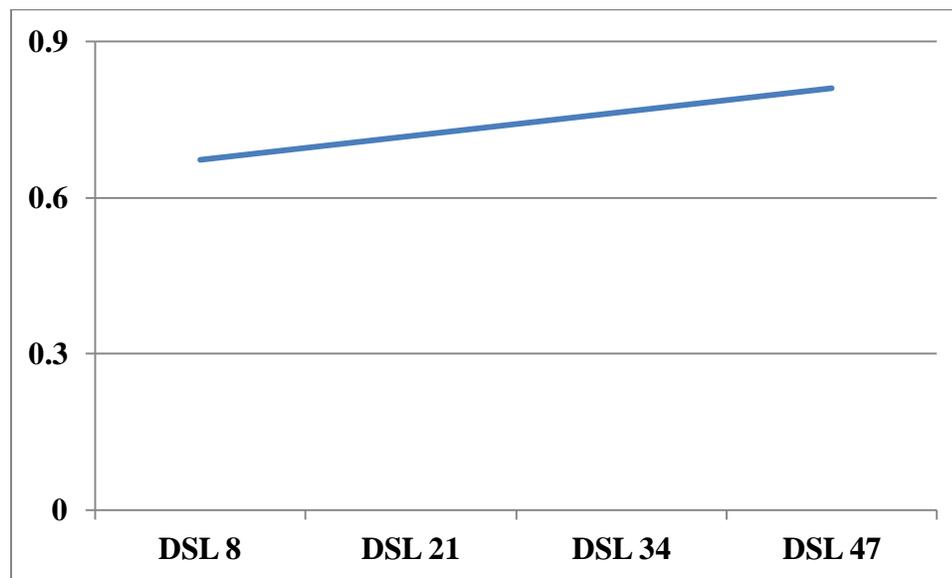
Figure 4.3. Relationship between Distal and Farrowing rate (FR).



Distal: the percentage of cells that had no cytoplasmic droplets on distal area.

According to Didion (2008), DSL had positive correlations with farrowing rate, however the correlation values were small (0.0147). In our research, we got a similar result. When DSL increased from 8 microns to 47 microns, FR was rose from 0.673 (about 67.3%) to 0.809 (about 81.0%). From our DSL data, if sperms move in a beeline, we can anticipate higher FR than others than from curved motion sperms. The mean and SD for DSL were 21.73 microns and 5.83, respectively (Table3).

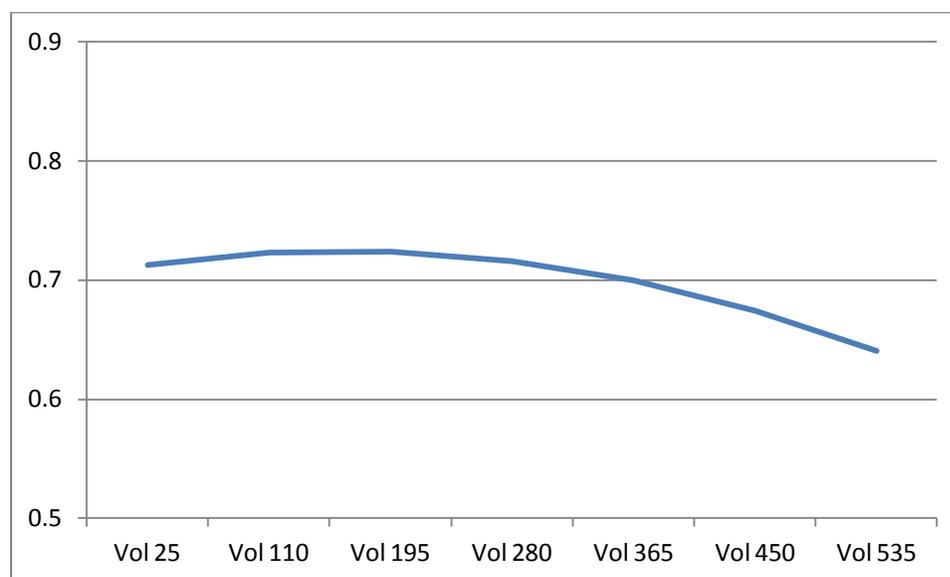
Figure 4.4. Relationship between DSL and Farrowing rate (FR).



Distance Straight Line (DSL): the distance (microns) that the sperm traveled in a straight line from the first frame to the last frame of the analysis.

In the quadratic regression model, Figure 4.6 showed the relationship between Vol and FR. FR were increased from 0.7129 (about 71.3%) to 0.7236 (about 72.4%) when the semen Vol (Figure 4.6) was increased from 25 Mℓ to 195 Mℓ. However, FR was decreased from 0.7158 (about 71.6%) to 0.6402 (about 64.0%) when the semen Vol was increased from 280 Mℓ to 535 Mℓ. The mean value and standard deviation (SD) of Vol were 193.89 and 87.89, respectively.

Figure 4.5. Relationship between Vol and Farrowing rate (FR)^a.

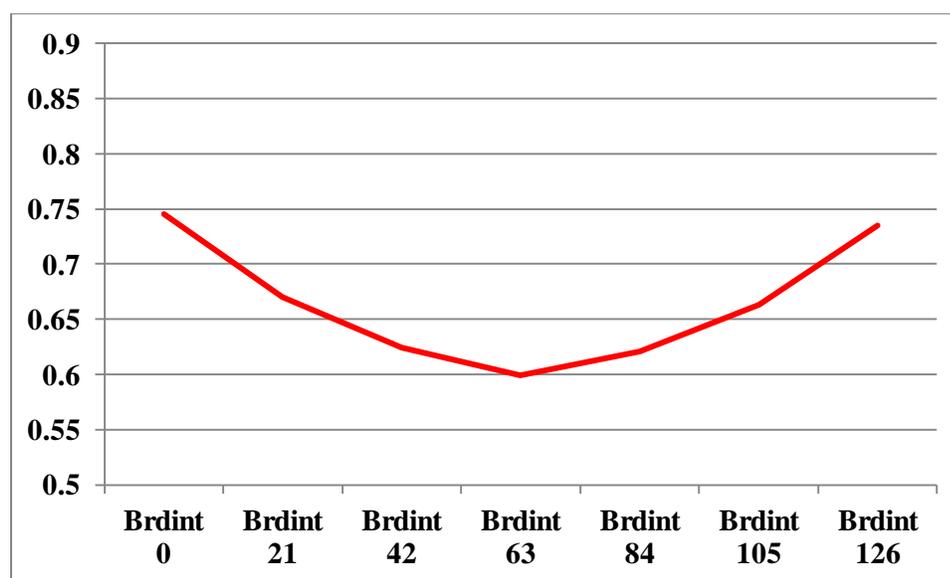


Semen volume (Vol): Total volume of the raw ejaculate expressed in milliliters (Mℓ).

^a Quadratic Regression

The Optimum of FR was located in both extremes of Brdint and intermediate Brdint variables had lower FR. The mean and SD of Brdint were 5.301 (about 5.3 day) and 15.637 (about 15.6 days), respectively. Brdint values had huge variations because some sows had re-estrus problems.

Figure 4.6. Relationship between Brdint and Farrowing rate (FR)^a.



Brdint: breeding interval.

^a Quadratic Regression

Total born (TB)

In STEPWISE, MAXR and R-square analysis, Vol, Con, VAP, AOC, Compos, Tail and Proximal were chosen for TB. Linear regression model for TB was,

$$Y (TB) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Con} + \text{VAP} + \text{AOC} + \text{Compos} + \text{Tail} + \text{Proximal} + \text{error } (\epsilon),$$

Vol, Compos and Tail were significant in the linear regression model for TB ($P < 0.1$). However, Con, VAP, AOC and Proximal did not indicate significant P-value, so they were eliminated from quadratic regression models (Table 6). Quadratic model for TB was,

$$Y (TB) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Compos} + \text{Tail} + \text{Vol}^2 + \text{Compos}^2 + \text{Tail}^2 + \text{error } (\epsilon),$$

However, no semen traits were significant in quadratic regression model (Table 6).

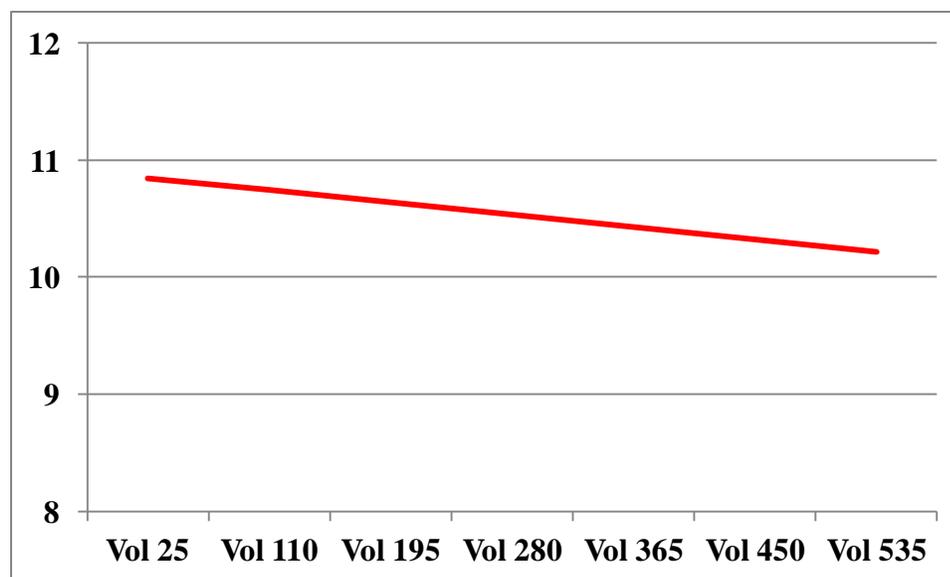
Table 6. Linear and quadratic regressions (b) and standard errors (se) and P-values from final model of Total born (TB).

Linear regressions			Linear and quadratic regressions		
	b±se	P-value		b±se	P-value
Brdint, day	-0.00868±0.003963	0.0286	Brdint, day	-0.00857±0.003965	0.0306
Brdint2	0.000044±0.000045	0.3288	Brdint2	0.000043±0.000045	0.3424
Boarage, day	0.001273±0.000395	0.0013	Boarage, day	0.001264±0.000392	0.0013
Drest, day	0.009455±0.008416	0.2613	Drest, day	0.008192±0.008403	0.3297
Vol, Mℓ	-0.00123±0.000484	0.011	Vol, Mℓ	-0.00086±0.001408	0.5422
Compos	0.03171±0.005504	<.0001	Compos	0.04363±0.04680	0.3512
Tail, %	-0.04005±0.02111	0.0579	Tail, %	0.8477±0.7564	0.2624
			Vol2	-1.27E-7±2.776E-6	0.9636
			Compos2	-0.00012±0.000294	0.6712
			Tail2	-0.00456±0.003916	0.2441

Brdint: breeding interval, Boarage: semen collecting date minus boar birth date, Drest: semen collection interval in AI center, Vol: raw semen volume, Compos: composite score, Tail: the percentage of normal tail.

Just like the quadratic result for FR, in the linear regression model, when the Vol was increased from 25ml to 535ml, TB was decreased from 10.84 (about 10.8 head) to 10.21 (about 10.2 head). We still do not understand the reason. One thing is for sure: the higher raw semen quantities do not mean a number of sperm in each semen collection. Another possibility is that excess or lack of unknown semen materials which were involved with accessory glands of male reproductive system can make these undesirable results. The mean and SD of Vol were 193.89 Mℓ and 87.29, respectively.

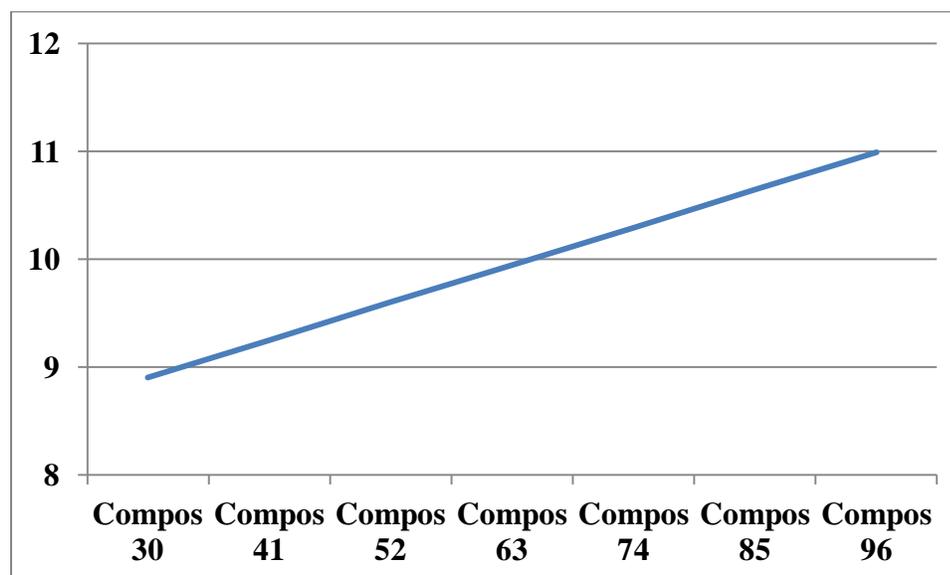
Figure 5.1. Relationship between Vol and Total born (TB).



Semen volume (Vol): Total volume of the raw ejaculate expressed in milliliters (Mℓ).

We did not find any researcher in which higher semen Compos provided desirable reproduction performance. However, the score calculation was based on the percentage of normal motility and morphology. Thus, we anticipated that higher Compos would offer positive effects to the reproduction performance. In our data, when Compos went up from 30 to 96, the number of total born was also increased from 8.90 (about 8.9 head) to 10.29 (about 10.3 head). The mean and SD of Compos were 84.71 and 7.49, respectively.

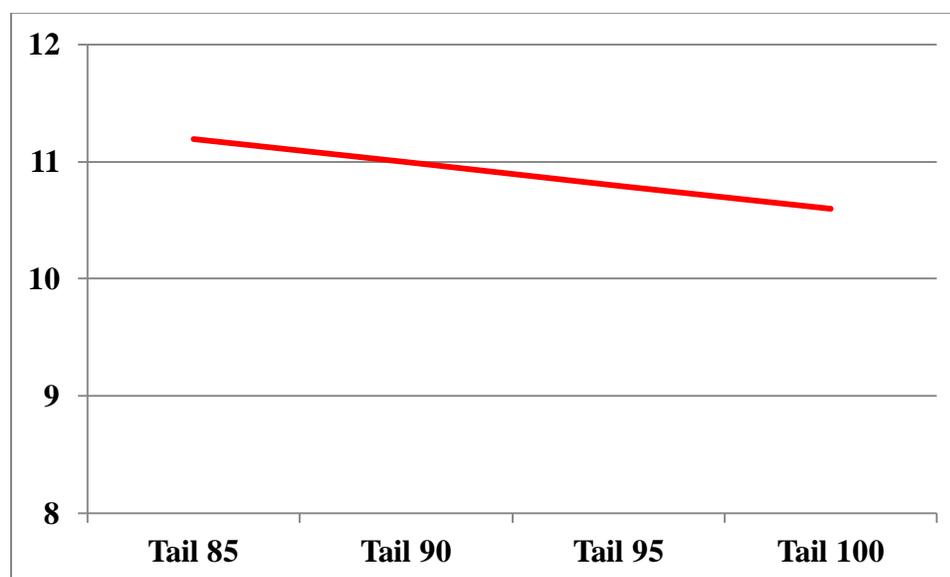
Figure 5.2. Relationship between Compos and Total born (TB).



Composite score (Compos): The product of % Motile multiplied by % Normal (Normal determined by normal morphology. Sperm have no abnormal head and tail, and cytoplasmic droplets) multiplied by % Viable (Viable is determined by multiplying Total Cells (live and dead) by % Motile by % Normal).

One of the most surprising results was the relationship between the percentage of normal sperm tail and TB. Before the analysis, we had no doubt Tail would have a positive regression value (b), however it was negative. TB decreased from 11.19 (about 11.2 head) to 10.59 (about 10.6 head) when the percentage of normal sperm tail was increased from 85% to 100%. The mean and SD of Tail were 98.96% and 1.67, respectively.

Figure 5.3. Relationship between Tail and Total born (TB).



Tail: the percentage of normal tail.

Stillborn (SB)

In STEPWISE, MAXR and R-square analysis, Vol, Con, Tmot, Head, Proximal, Compos, Distal, Tail and AOC were chosen for SB.

$$Y (SB) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Vol} + \text{Con} + \text{Tmot} + \text{Head} + \text{Proximal} + \text{Compos} + \text{Distal} + \text{Tail} + \text{AOC} + \text{error } (\varepsilon),$$

Vol and Con had non-significant P-values ($P > 0.1$) in linear regression model, so they are eliminated in quadratic regression model. Quadratic model for SB was,

$$Y (SB) = \mu + \text{Farm} + \text{Dline} + \text{Brdint} + \text{Brdint}^2 + \text{Parity} + \text{Sow} + \text{Boar} + \text{Sery} + \text{Serm} + \text{Boarage} + \text{Drest} + \text{Tmot} + \text{Head} + \text{Proximal} + \text{Compos} + \text{Distal} + \text{Tail} + \text{AOC} + \text{Tmot}^2 + \text{Head}^2 + \text{Proximal}^2 + \text{Compos}^2 + \text{Distal}^2 + \text{Tail}^2 + \text{AOC}^2 + \text{error } (\varepsilon),$$

However, no semen characteristics were significant in the quadratic model (Table 7).

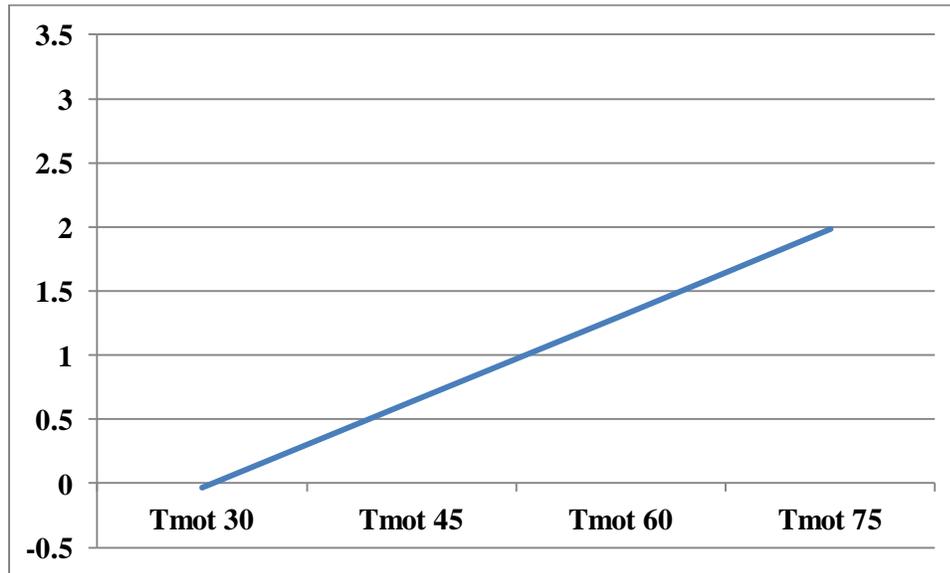
Table 7. Linear and quadratic regressions(b) and standard errors (se) and P-values from final model of Stillborn (SB).

Linear regressions			Linear and quadratic regressions		
	b±se	P-value		b±se	P-value
Brdint, day	-0.00181±0.002116	0.3922	Brdint,day	-0.00185±0.002117	0.3819
Brdint2	0.000051±0.000024	0.0357	Brdint2	0.000051±0.000024	0.0341
Boarage, day	0.000262±0.000195	0.1796	Boarage, day	0.000221±0.00019	0.2432
Drest, day	0.002726±0.004458	0.5408	Drest, day	0.002478±0.004454	0.578
Tmot, %	0.03358±0.01591	0.0349	Tmot, %	-0.00994±0.07668	0.8969
Head, %	0.05235±0.02158	0.0153	Head, %	-1.6765±1.4593	0.2506
Proximal, %	0.03501±0.01852	0.0588	Proximal, %	-0.01294±0.2324	0.9556
Compos, %	-0.02706±0.01614	0.0936	Compos, %	0.02426±0.07672	0.7518
Distal, %	0.01532±0.009083	0.0917	Distal, %	-0.01612±0.2267	0.9433
Tail, %	0.04625±0.01706	0.0067	Tail, %	-0.2954±0.4099	0.4712
AOC, degree	-0.00581±0.002609	0.0259	AOC, degree	-0.00207±0.007548	0.784
			Tmot2	0.000255±0.000437	0.559
			Head2	0.008816±0.00744	0.2361
			Proximal2	0.000236±0.00122	0.8468
			Compos2	-0.00031±0.000447	0.4827
			Distal2	0.000163±0.001185	0.8908
			Tail2	0.001766±0.002116	0.4039
			AOC2	-0.00009±0.000172	0.5838

Brdint: breeding interval, Boarage: semen collecting date minus boar birth date, Drest: semen collection interval in AI center, Tmot: total motility, Head: the percentage of normal head, Compos: composite score, Distal: distal cytoplasmic droplet, Tail: the percentage of normal tail, AOC: Average orientation change.

We had some odd regression value in Tmot 30. When Tmot increased from 30 to 75, SB also increased from -0.034 to 1.981 (about 1.98 head).

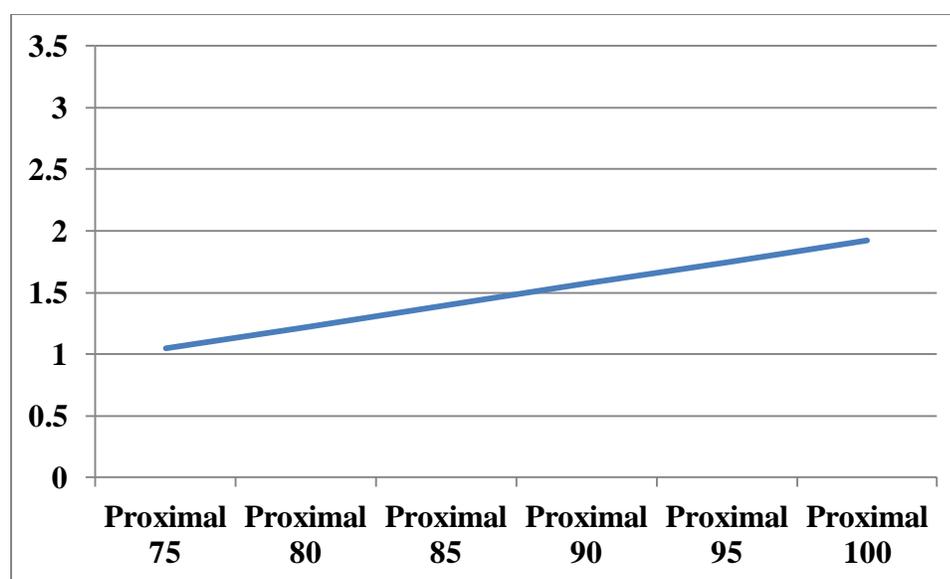
Figure 6.1. Relationship between Tmot and Stillborn (SB).



Total Motility (Tmot): the percentage of spermatozoa that had any movement of the sperm head.

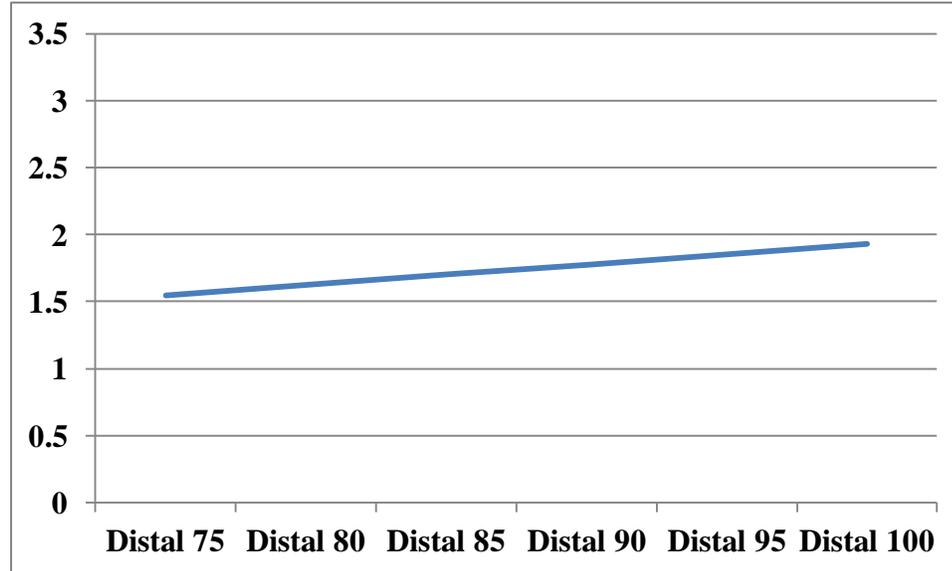
When the percentage of proximal and distal were increased, SB was also increased (Figure 6.2 and Figure 6.3). The mean values of Proximal and Distal were 99.30 and 97.82, respectively. I could not find any research reporting the correlation between cytoplasmic droplets and SB in swine. A number of researches mentioned that proximal and distal cytoplasmic droplets might compromise FR, not SB. According to Waberski et al. (1994), high percentage of proximal and distal cytoplasmic droplets had negative correlation with pregnancy rate and litter size.

Figure 6.2. Relationship between Proximal and Stillborn (SB).



Proximal : the percentage of cells that had no cytoplasmic droplets on proximal area.

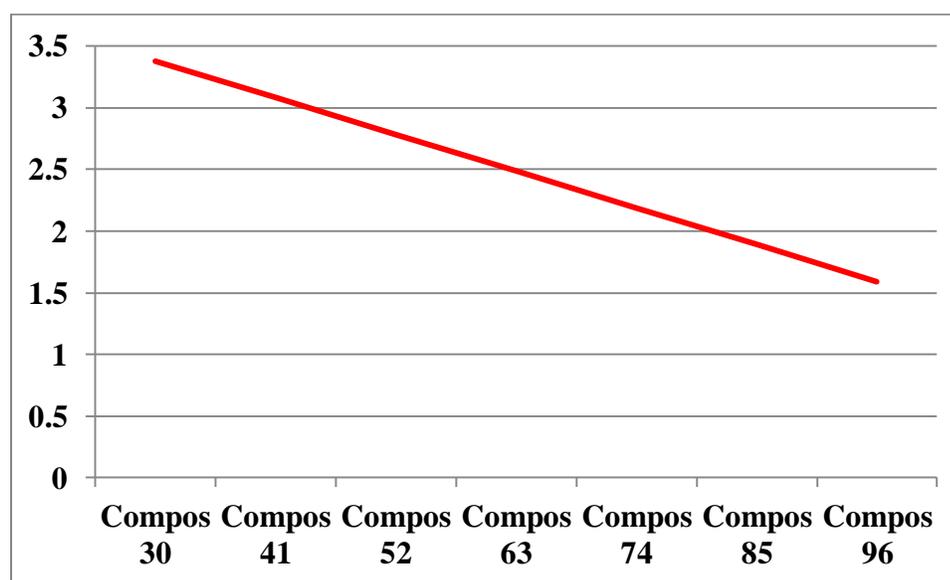
Figure 6.3. Relationship between Distal and Stillborn (SB).



Distal: the percentage of cells that had no cytoplasmic droplets on distal area.

When the Compos value (Figure 6.4) was increased from 30 to 96, SB was decreased from 3.377 (about 3.4 head) to 1.591 (about 1.6 head). It maybe a inevitable result because if semen had higher percentage of normal morphology and motility, it would likely give positive impetus to fertilization. The mean and SD of Compos were 84.71 and 7.49, respectively.

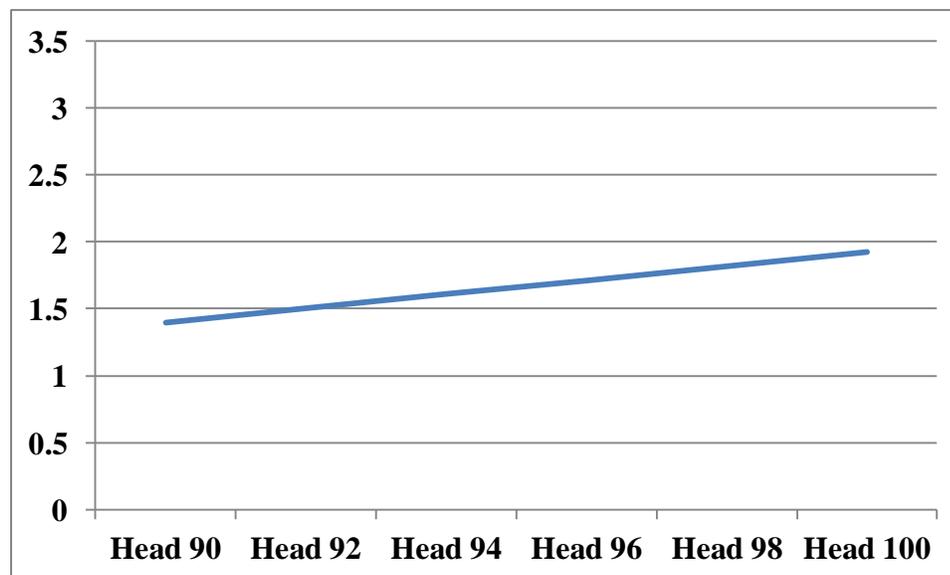
Figure 6.4. Relationship between Compos and Stillborn (SB).



Composite score (Compos): The product of % Motile multiplied by % Normal (Normal determined by normal morphology. Sperm have no abnormal head and tail, and cytoplasmic droplets) multiplied by % Viable (Viable is determined by multiplying Total Cells (live and dead) by % Motile by % Normal).

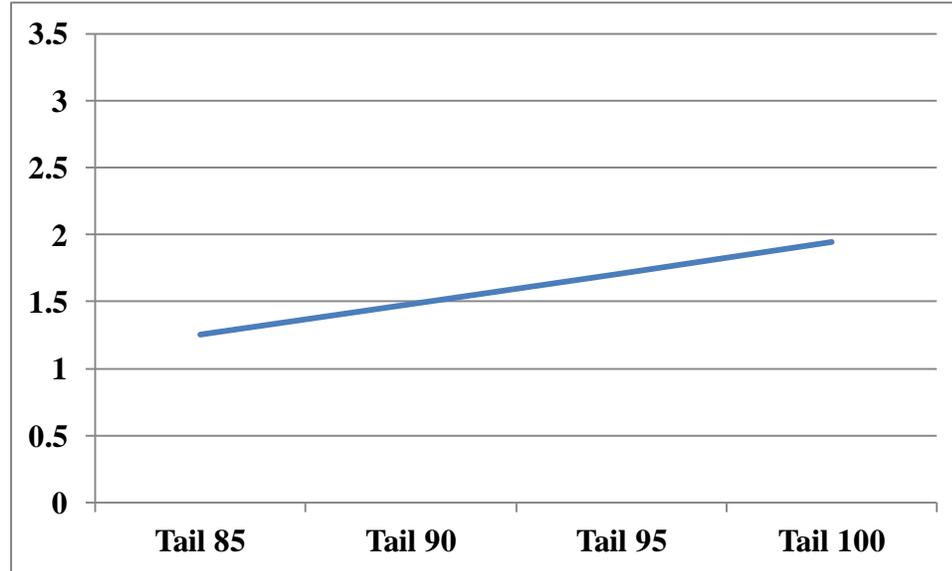
In Figure 6.5., when the normality of sperm head increased from 90 to 100, SB also increased from 1.398 (about 1.4 head) to 1.712 (about 1.7 head). In Figure 6.6., when the normality of sperm tail increased from 85 to 100, SB increased from 1.251 (about 1.3head) to 1.945 (about 1.9 head). The mean values of Head and Tail were 99.53 and 98.96, respectively. I do not fully understand why when the normality of Head and Tail increased, SB also increased. If the normality of head or tail was also related with increasing TB, we could explain it, because SB is usually increased when TB is increased. However, the Head had no statistically significant effect to TB, and the Tail had negative regression value in TB.

Figure 6.5. Relationship between Head and Stillborn (SB).



Head: the percentage of normal head.

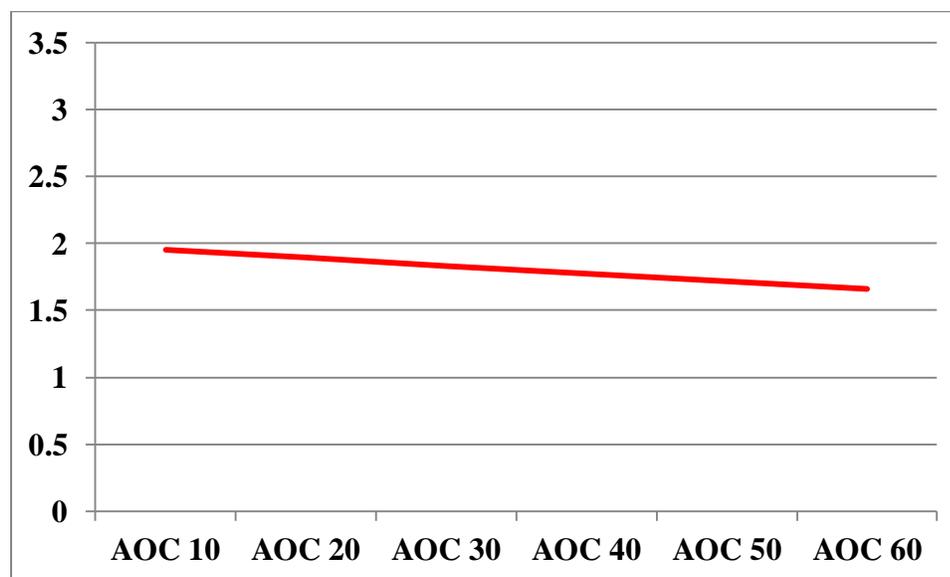
Figure 6.6. Relationship between Tail and Stillborn (SB).



Tail: the percentage of normal tail.

AOC had negative regression value in SB. SB was decreased from 1.950 (about 2.0 head) to 1.659 (about 1.7 head) when AOC was increased from 10 to 60. In other words, if sperm head changed their direction in large angle, SB would be decreased.

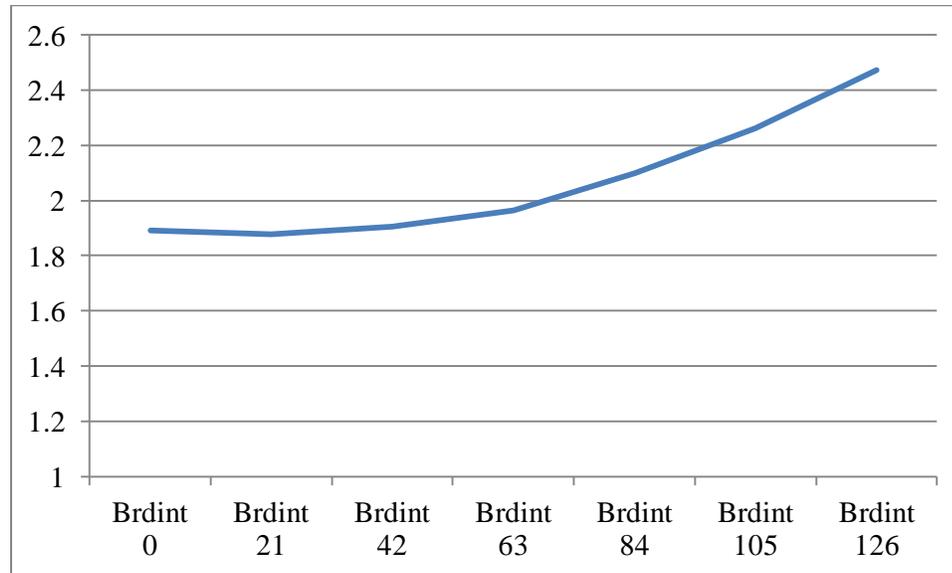
Figure 6.7. Relationship between AOC and Stillborn (SB).



Average orientation change (AOC): the average change in orientation of the head of the sperm cell between frames during the measurement period measured in degrees.

From Brdint 0 to Brdint 42, SB changed little. However, after Brdint63, SB increased until Brdint 126. The mean and SD of Brdint were 5.301 (about 5.3 day) and 15.637 (about 15.6 days), respectively.

Figure 6.8. Relationship between Brdint and Stillborn (SB).



Brdint : Breeding interval

Chapter 5: Discussion

As an industrial animal, pigs are outstanding animal among livestock. Pigs are one kind of fructuous animals and they produce high quality meat. And also, they have relatively short generation interval. Most quantitative traits of pigs, such as litter size, the number of born alive, the number of weaning piglet, average daily gain and carcass weight are economically important. AI plays a vital role in swine genetic improvement and it will be a great contribution to the future research. In the past, many semen or sperm-related studies were just focused on semen volume, concentration, sperm motility and morphology, however, the advancement of computers and optical instruments provides more detail information about semen or sperm traits. Unfortunately, there are not much research done in a same manner and many of them are not compatible with others.

The STEPWISE, MAXR and R-square were used to select semen traits to include in mixed models. An attempt to use PROC GLINMMIX of SAS for binomial traits to analyze FR was made; however SAS showed “out of memory” message and stopped the process – the data set was huge. Thus, final analyses were done with the PROC MIXED method of SAS.

The semen characteristics initially selected to include in models of FR were Tmot, STR, VAP, Vol, DCL, Distal and DSL. However, the P-values of STR and DCL were over our threshold ($P > 0.10$), so they were not included in the quadratic regression model. Tmot, VAP, Vol, Distal and DSL were significant in linear regression model only and Vol was significant ($P < 0.10$) in the quadratic model.

For TB, STEPWISE, MAXR and R-square methods showed that Vol, Con, VAP, AOC, Compos, Tail and Proximal needed to be included in linear regression model. Vol, Compos and Tail had significant P-values ($P < 0.10$) in linear model, but they were not significant in quadratic.

For SB, Tmot, Vol, Con, Head, Proximal, Compos, Distal, Tail and AOC were selected as semen characteristics to include in mixed models. Vol and Con were eliminated for quadratic analysis because their linear regression result was not significant ($P > 0.10$). Quadratic effects of Tmot, Head, Proximal, compos, Distal, Tail and AOC also were not significant.

In FR and TB results, we were surprised that when semen Vol was increased, FR was generally decreased (Figure 4.6) and TB also declined (Figure 5.1). The mean value of Vol was 193.89 mL (Table 3.). If SpermVision® System did not measure sperm Con, Con should be suspected as the reason of low FR and TB, however, the system measured Con and sperm numbers for each AI dose are automatically calculated. We cannot fully understand the reasons, but one thing is for sure: the largest amounts of raw semen do not mean mass quantities of spermatozoa. Excess or lack of unknown semen materials of large quantities of semen could be another possibility of decreasing FR and TB.

According to the previous research about VAP, decreasing VAP was associated with higher conception rate (Holt et al., 1997). However, according to Didion (2008), VAP had positive correlations with FR and according to Hirai et al. (2001) VAP and TB had a positive correlation. Our study affirmed that if the AI sperm have high VAP value, we get low FR, and we could not find the correlation between VAP and TB.

Only one reference existed to explain the relationship of DSL and FR. According to Didion (2008), DSL had positive correlations with FR. Findings were consistent with that result (Figure 4.5).

Existing proximal and distal droplets reduced farrowing rate and litter size in swine (Waberski et al.; Feitsma et al.). In our research, only in linear regression model was a relationship between Distal and FR (Figure 4.3) and between Distal and SB (Figure 6.3) detected. In SB, the relationship between Proximal and SB were observed. However, they did not have any significant P-values in quadratic regression models. For FR, this result was unusual. However, if this situation was happening repeatedly later, we need to rethink about sperm maturation effects for AI semen extender. A number of researches mentioned that proximal and distal cytoplasmic droplets might compromise FR, not SB. However, our results for Proximal and Distal revealed that when spermatozoa had less Proximal and Distal, the number of SB increased.

One surprise result was the relationship between the percent normal sperm tail and TB. Before the analysis, a higher percentage of normal Tail was expected to have a positive regression value (b) with TB, however it moved the opposite way. And also, in SB, when the normality of Head and Tail increased, SB also increased. We still cannot clearly explain the reason. If the normality of head or tail was also related with increasing TB, we could be explain it. Because SB is usually increased when TB is increased. However, the Head did not indicate statistically significant effects to the TB, and the Tail had negative regression value in TB.

From our result for FR, Tmot and FR had positive relationship. It was clearly anticipated before the analysis. In SB result, some odd regression value for Tmot removed. Tmot and SB had positive relationship, a result not anticipated. Pmot (the percentage of spermatozoa which moved in a forward direction.) and Lmot (the percentage of spermatozoa that are alive, but move very little in the forward direction.) were not shown as statistically significant effects in any reproduction performances.

Semen composite score (Compos) showed statistical significance in linear regression for TB and SB ($P < 0.10$). We could not find any research that Compos was related with desirable reproduction performance, however, before the analysis, we anticipated that higher Compos would offer positive effects to reproduction performance. Compos had positive relationship with TB, however Compos showed negative relationship with SB. It might be an inevitable results because if semen had higher percentage of normal morphology and motility, it would likely give positive impetus to fertilization.

We could not find research which revealed the relationship between AOC and reproduction performance. In our result, AOC had negative regression value on SB. In other words, if sperm head changed their direction in large angle (Figure 3), SB would be decreased.

From the quadratic regression result of FR (Figure 4.7), the Optimum of FR was located in both extremes parts of Brdint. And according to the quadratic result of SB (Figure 6.8), when Brdint increased, SB showed significant boosts. Increasing Brdint would have bad effects to the non-productive female days, rotating rate of sow and the number of still

born. Extremely increased Brdint could lead to a revival of FR, however, it was certainly doing more harm than good in SB and farm performances.

The concepts for detail semen traits analysis were established before the digital equipments developed. However, in swine, the detail methods to find ideal semen characteristics are still not developed well. We still cannot affirm which semen characteristics are important or not because there are few research papers existing. Some of them mentioned opposite results to each other. We found some research papers which were related with human semen traits; however they were not appropriate to explain boar semen characteristics. And also, each measurement method is not compatible with other methods. We need to overcome these situations from the further research.

Chapter 6: Literature Cited

1. Alm K, Peltoniemi O, Koskinen E, Andersson M, 2006. Porcine field fertility with two different insemination doses and the effect of sperm morphology. *Reprod Dom Anim* 41, 210-213.
2. Boerke A, Tsai PS, Garcia-Gil N, Brewis IA, Gadella BM , 2008. Capacitation-dependent reorganization of microdomains in the apical sperm head plasma membrane: functional relationship with zona binding and the zona-induced acrosome reaction. *Theriogenology* 70:1188–1196
3. Broekhuijse MLWJ, Sostaric E, Feitsma H and Gadella BM, 2012. Application of computer-assisted semen analysis to explain variations in pig fertility. *J. ANIM SCI*, 90:779-789.
4. Cummins JM and Woodall PF, 1985. On mammalian sperm dimensions. *J. Reprod Fertil* 75,153–175.
5. Didion BA, 2008. Computer-assisted semen analysis and its utility for profiling boar semen samples. *Theriogenology*, Vol. 70, Issue8, 1374-1376.
6. Feitsma H, Bergsma R, Ducro-Steeverink DW, 2006. The effect of morphological abnormal cells on sow fertility. In: *Proceedings of the 19th IPVS congress*; Abstract No. P. 35-11.
7. Flowers WL, 1996. Semen evaluation, extension, packaging and transportation methods. *Proc AASP*. 469–482.
8. Ford WCL, 2006. Glycolysis and sperm motility: does a spoonful of sugar help the flagellum go round? *Human Reproduction Update*, Vol.12, No.3 pp. 269–274.

9. Gadea J, Matas C, Lucas X, 1998, Prediction of porcine semen fertility by homologous in vitro penetration (hIVP) assay. *Animal Reproduction Science*, 56: 95–108.
10. Gadea J, 2005. Sperm factors related to in vitro and in vivo porcine fertility. *Theriogenology* 63:431-444.
11. Gomendio M, Malo AF, Soler AJ, Fernandez-Santos MR, Estes MC, Garcia AJ, Roldan ERS & Garde J, 2006b. Male fertility and sex ratio at birth in red deer. *Science* 314 1445–1447.
12. Hirai M, Boersma A, Hoeflich A, Wolf, J. Foll E, Aumuller TR, and Braun J, 2001. Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): relation to fertility and seminal plasma growth factors. *J. Androl*, 22:104–110.
13. Holst SJ, 1949. Sterility in boars. *Nord Vet Med.* 1:87-120.
14. Holt C, Holt WV, Moore HD, Reed HC, Curnock RM, 1997. Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. *J. of Andrology*, Vol. 18, No.3
15. Ko JCH, Evans LE, Althouse GC, 1989. Toxicity effects of latex gloves on boar spermatozoa. *Theriogenology* 31:1159-1164.
16. Kondracki, S, 2003. Breed differences in semen characteristics of boars used in artificial insemination in Poland, *Pig News Inf.*, 24: 119N-122N.
17. Liu DY, Clarke GN, Baker HW 1991, Relationship between sperm motility assessed with the Hamilton-Thorn motility analyzer and fertilization rates in vitro. *J Androl*; 12: 231-9.

18. Lopez-Fernandez C, Perez-Llano B, Garcia-Casado P, Sala R, Gosalbez A, Arroyo F, Fernandez JL, Gosalvez J, 2008. Sperm DNA fragmentation in a random sample of the Spanish boar livestock. *Animal Reproduction Science* 103, 87–98.
19. Morgan Morrow, 1998. Guidelines for cytoplasmic droplets, *Swine News* July, Volume 21, Number 6.
20. Rodriguez-Martinez H, Ekstedt E, Einarsson S, 1990. Acidification of the epididymal fluid in the boar. *Int. J. Androl.* 13, 238-243.
21. Rozeboom KJ, 2000. Evaluating Boar Semen Quality. *Animal science facts* ANS00-812S.
22. Saacke, RG, Nadir S, Nebel, RL, 1994. Relationship of semen quality to sperm transport, fertilization and embryo quality in ruminants. *Theriogenology* 41, 45–49.
23. Shipley C, 1999. Breeding soundness examination in the boar. *Swine Health Prod* 7, 117–120.
24. Tardii S, Laforest J. -P, Cormier N. and Bailey JL, 1999. The importance of porcine sperm parameters on fertility in vivo. *Theriogenology* 52:447–459.
25. Waberski D, Meding S, Dirksen G, Weitze KF, Leiding C, Hahn R, 1994. Fertility of long-term-stored boar semen: Influence of extender (Androhep and Kiev), storage time and plasma droplets in the semen. *Anim Reprod Sci.* 36:145–151.
26. WHO, 2010. WHO laboratory manual for the examination and processing of human semen. 5th ed., Geneva.