RESEARCH REPORT

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ANIMAL SCIENCE

Title: Relationships between Seminal Plasma Proteins and Boar Fertility: Development of a Proactive Semen Fertility Test (Renewal – Year 2)
NPB #98-097

Investigator: W.L. Flowers

Institution: North Carolina State University
Raleigh, N.C.

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Abstract

Results from the first year of the proposal demonstrated that concentrations of 2 seminal plasma proteins were highly correlated with both in vivo and in vitro fertility of boars. These studies were performed on a small population (n=10) of boars. The objective of the second year of the study was to determine if the relationship between these 2 seminal plasma proteins and semen fertility existed for a much larger population of boars currently in use within commercial swine operations. Semen was collected weekly from boars in 2, 200-head, boar studs for 1 year. Concentrations of the 2 seminal plasma proteins (26 kDa, pI 6.2 and 55 kDa, pI 4.5) were determined for each collection. Farrowing rates and number of pigs born alive were recorded for sows inseminated with doses made from each ejaculate.

In general, there was a positive relationship between fertility and concentrations of the 2 seminal plasma proteins. Ejaculates with the highest levels of these proteins (> 10 relative units) exhibited the highest farrowing rates (86.7 $\pm$ 3.4 %) and greatest number of pigs born alive (11.2 $\pm$ 0.3) compared with those with lower levels (7.5 – 9.9 relative units, farrowing rate = 78.4 $\pm$ 3.1%, number of pigs born alive = 10.4 $\pm$ 0.3; 5.0 – 7.4 relative units, farrowing rate = 71.3 $\pm$ 3.9%, number of pigs born alive = 9.5 $\pm$ 0.3). These data demonstrate that quantification of these two proteins in seminal plasma holds promise for use in the development of a proactive semen fertility test.

Unfortunately, the range in farrowing rate and number of pigs born alive for ejaculates with the same concentration of these proteins was large - > 10 relative units – 80.0 to 94.0% and 10.2 to 12.2 pigs; 7.5 – 9.9 relative units – 70.2 to 86.0% and 8.8 to 11.2 pigs; 5.0 to 7.4 relative units – 65.4 to 80.3% and 7.8 to 10.7 pigs. This variation was primarily the result of individual differences among boars. In 380 of the 400 boars studied,

as the level of these two proteins decreased, farrowing rate and litter size also decreased. However, for some boars whose ejaculates contained 10 relative units farrowing rates were consistently high (> 90%), while for others with similar concentrations farrowing rates were low (< 80%). Consequently, these data indicate that while concentrations of these 2 seminal plasma proteins can be used to provide a qualitative rank for the boar fertility, their usefulness in terms of predicting actual quantitative levels of fertility is limited.

Introduction

During the first year of this grant, evaluations of the qualitative relationship between seminal plasma protein profiles and fertility of boars was completed. These data indicated that the relative concentrations of two seminal plasma proteins were highly correlated with in vitro fertility of boar semen and could be used to rank boars in terms of their fertility when bred to sows. The latter observation was determined by pooling semen from two boars and determining which one sired most of the pigs in a litter. Results from the first year of the grant demonstrated that monitoring concentrations of two seminal plasma proteins in semen has potential for being further developed into a proactive semen fertility test.

However, in order for this to occur, at least two other pieces of information about the relationships between concentrations of these proteins and boar fertility needs to be determined. First, a larger population of boars, preferably managed under commercial conditions, should be examined. This is important because the initial studies with the seminal plasma proteins were conducted on only 10 boars from the same genetic base. Second, the quantitative nature of the relationship between concentrations of seminal plasma proteins and farrowing rate and litter size needs to be elucidated. In other words, it is important to determine whether a specific concentration or amount of these proteins in seminal plasma consistently results in a given farrowing rate or litter size. If this is true, then measurement of these proteins in semen proactively could serve as a powerful management tool in genetic and breeding decisions. If it is not true, then monitoring of the proteins will still be useful for ranking boars in terms of their fertility, but primarily within an operation and not across different genetic lines or production environments. The primary objective of the second year of funding was to determine to determine the relationship between 2 seminal plasma proteins and semen fertility on commercial swine operations using A.I.

Objective

1). To determine the relationship between 2 seminal plasma proteins and semen fertility on commercial swine operations using A.I.

Experimental Procedures

Two large commercial swine operations with 200-head boar studs were used in the study. One boar stud contained 4 different genetic lines of boars, while the second one housed 3 distinct genotype of boars. Each stud supplied semen for 10 different 1500-sow operations. Every week for a period of 1 year (52 weeks), seminal plasma samples were obtained from all the boars collected on Monday, Tuesday and Thursday. The average number of samples obtained each week from the boar studs were 105 $\pm$ 2 and 98 $\pm$ 3,

respectively. Seminal plasma samples were frozen immediately after collection and were analyzed at a later date. The percentage of motile and morphologically normal spermatozoa were recorded for each ejaculate. Homospermic insemination doses consisting of 4 billion spermatozoa in a volume of 75 ml were made from each ejaculate. The average number of insemination doses made from each ejaculate was 15 $\pm$ 2. Sows received an insemination dose from a single boar once each day of estrus (homospermic mating regimen) throughout the duration of the study. The average number of sows bred with semen from each ejaculate was 8 $\pm$ 1. The date, time, a mating score and the breeding technician were recorded for each mating. When it was practically possible, the same breeding technician administered the all matings to a given sow. At the end of the study, farrowing rates and litter size were recorded from 84,448 sows bred from semen produced from 400 boars.

The seminal plasma protein profile of each ejaculate was determined using biochemical procedures (one- and two-dimensional polyacrylamide gel electrophoresis with isoelectrical focusing and densitometry).[1] Two types of statistical analyses were conducted. First, analysis of variance procedures will be used to examine differences among boars in terms of farrowing rate, number of pigs born alive and concentrations of seminal plasma proteins. The model consisted of boar, ejaculate, time, semen age, farm, breeding technician and appropriate interactions. The second analyses used regression procedures (stepwise; frontward and backward) to determine the relative importance of boar, time, ejaculate, seminal plasma protein concentration, farm and breeding technician on farrowing rates and litter size. An important aspect of the experimental design of this project was that comparisons be made across different farms. Theoretically, an ideal fertility test should be able to predict relative difference among boars when the boars are compared in a number of different production environments. Consequently, it is important to note that data from all farms were used in this study.

Results

The relationship between the seminal plasma protein profile and boar fertility as measured by farrowing rates and number of pigs born alive is shown in Table 1. In general, as the concentrations of the 26 kDa, pI 6.2 and the 55 kDa, pI 4.5 proteins increased, so did farrowing rate and litter size ($p < .05$). Unfortunately, the range in values for farrowing rate and number of pigs born alive from ejaculates with similar seminal plasma protein concentrations was large. This variation was primarily due to individual variation among boars and not among ejaculates from the same boar. In other words, for any given boar in the study, ejaculates that contained 10 relative units of these seminal plasma proteins always produced farrowing rates and litter sizes greater than their counterparts that contained lower concentrations. In contrast, for seminal plasma that contained 10 relative units of these proteins from two different boars, farrowing rates and litter sizes often differed, in the extreme case, by 14% and 2 pigs, respectively. As a result, quantification of the seminal plasma protein profile appears to be an excellent qualitative test for ranking boars prospectively in terms of their subsequent fertility. However, it does not appear that this information can be used, by itself, to predict the actual farrowing rates and litter sizes that a producer should expect from individual animals.

It is important to remember that in the present study, ejaculates that did not meet minimum criteria for morphology (> 70% normal spermatozoa) and motility (> 60% motile spermatozoa) were discarded and not used for breeding. Consequently, at least in this

study, a significant proportion of ejaculates, 33% (3447/10590), passed these microscopic analyses, yet produced suboptimal farrowing rates and litter sizes. Because the study was conducted in a commercial setting, it is tempting to speculate that as many as one-third of the ejaculates produced which are judged to be acceptable via microscopic tests are, in reality, subfertile. These ejaculates were ones that contained, on the average, less than 7.5 relative units of the seminal plasma fertility proteins (26 kDa, pI 6.2 and the 55 kDa, pI 4.5 proteins). It was also interesting to note that the frequency of these types of ejaculates – ones that passed motility and morphology tests, but resulted in poor fertility was increased for certain boars during the summer months. One interpretation of this observation is that there was a population of boars, in the present study, which exhibited increased sensitivity to the climatic conditions associated with the summer months. If this is, in fact, what occurred, then the physiological effects of this environmental condition, obviously did not manifest itself in these boars via changes in semen morphology and/or motility. Additional studies are required to verify these speculations.

Table 1. Relationship between Seminal Plasma Protein Profile and Fertility in Boars (mean $\pm$ S.E.)

Seminal Plasma Protein Concentration (relative units)[a]	Number of Ejaculates	Farrowing Rate (%)[b]	Number of Pigs Born Alive[b]
≥ 10	3,532	86.7 ± 3.4^{w} (80.0 – 94.0)	11.2 ± 0.3^{w} (10.2 – 12.2)
7.5 – 9.9	3,611	78.4 ± 3.1^{x} (70.2 – 86.0)	10.4 ± 0.3^{x} (8.8 – 11.2)
5.0 – 7.4	2,321	71.3 ± 3.9^{y} (65.4 – 80.3)	9.5 ± 0.3^{y} (7.8 – 10.7)
≤ 4.9	1,126	62.7 ± 4.0^{z} (54.6 – 77.1)	8.6 ± 0.4^{z} (7.2 – 10.0)

[a]relative concentrations of the 26 kDa, pI 6.2 and the 55 kDa, pI 4.5 proteins were added together to produce these categories.

[b]range of values for each variable are included in parentheses below the mean.

[w,x,y,z]means with different superscripts within each column differ ($p < .05$).

In summary, based on these results, quantification of two seminal plasma proteins appears to be an accurate proactive test for qualitative, but not quantitative assessments of semen fertility. In addition, it is conceivable that quantification of these two seminal plasma proteins could be used as an additional step to "screen" for ejaculates with reduced fertility which have passed commonly used microscopic criteria. This could prove to be critically important in regions with tropical or subtropical climatic conditions during portions of the year.

References

1. Flowers, W.L., Siggens, K., McLaren, D.G. 1996. J. Anim. Sci. 74, suppl. 1, 223.