Boar seminal plasma proteins and their relevance to reproductive technologies

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Seminal plasma proteins participate in a number of events important for fertilization and the establishment of pregnancy. As a result, attempts have been made to use them to enhance reproductive performance associated with several swine reproductive technologies. Inclusion of seminal plasma into cryopreservation and sex-sorting protocols improved sperm viability and membrane integrity and suppressed capacitation-like changes which are considered to be major challenges associated with these techniques. Unfortunately, it has yet to be shown that these improvements consistently increase in vivo fertility. In contrast, pre-breeding administration of seminal plasma in conjunction with conventional breeding regimens improved farrowing rates and numbers of pigs born alive on commercial farms that already had very good reproductive performance. The best way to capture these beneficial effects in A.I. programs currently is being investigated. Finally, three seminal plasma proteins appear to have reasonable correlations with fertility in boars that normally produce sperm with excellent motility and morphology. They hold potential for development of prospective male fertility tests. However, there is some evidence that indicates consideration of the complete profile of a boar's seminal plasma proteins may be more appropriate for this purpose as opposed to concentrating on individual ones independently. Preliminary results from a field study indicate that farrowing rate and litter sizes are superior in boars with high levels of two seminal plasma proteins associated with fertility compared with their counterparts in which only one of these is elevated. All of these technologies will benefit from continued research efforts devoted to the additional characterization of proteins in seminal plasma and elucidation of their biological effects on swine reproductive physiology.

Introduction

During natural matings, spermatozoa along with a small volume of fluid leave the cauda epididymi; are transported to the urethra where they are mixed with large volumes of secretions from the secondary sex glands; and then are deposited directly into the cervix of the sow.

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Consequently, for a number of years, seminal plasma was regarded primarily as a transport vehicle for sperm (Garner and Hafez, 1993). However, more recently, studies have demonstrated that the rich mixture of compounds in seminal plasma have profound effects on boar sperm; the sow's reproductive tract; and interactions between the two (Waberski, 1997; Robertson, 2007; Schuberth *et al*., 2008; Rodriquez-Martinez *et al*., 2009). As a result, seminal plasma, especially its protein component, is now believed to play an active role in fertilization and the successful establishment of pregnancy. This has stimulated an active area of research focused on determining whether specific proteins or seminal plasma, in general, can be used to enhance the effectiveness of reproductive technologies important to the swine industry.

These efforts have been concentrated in two main areas. The first one is based on the observation that the majority of swine reproductive technologies require that seminal plasma be diluted or completely removed. Examples include preparation of A.I. doses from sperm-rich fractions or whole ejaculates via the addition of semen extender (Johnson *et al*., 2000) and isolation of sperm for freezing, sex-sorting, and IVF protocols (Johnson *et al*., 2005; Gil *et al*., 2008; Parilla *et al*., 2009). Presumably, there are threshold levels for these proteins below which their biological effects become negligible. Replenishment of, or, perhaps, even enhancement with these proteins seem like physiologically reasonable approaches for improving fertility of females bred with semen produced via these procedures.

The second one is an attempt to establish prospective tests for boar fertility. This is a critical need because use of pooled semen is common throughout much of the swine industry (Knox *et al*., 2008) and it is clear that boars with normal levels of motility and morphology can differ considerably in terms of the ability of their sperm to produce live pigs (Flowers, 1997; Xu *et al*., 1998; Ruiz-Sanchez *et al*., 2006; Flowers, 2008). Development of prospective screening tools that can detect fertility differences among boars with normal semen characteristics would allow for the use of only the best boars which, in turn, should result in increased selection pressure on other economically important traits. It has been suggested that seminal plasma proteins have potential for accomplishing this goal (Foxcroft *et al*., 2008; Flowers, 2009; Dyck *et al*., 2011).

The primary objective of this review is to examine the use of seminal plasma proteins for enhancement of reproductive technologies such as artificial insemination with fresh, frozen, or sex-sorted semen and development of prospective boar fertility tests. Special attention will be given to studies that have evaluated their effects on farrowing rates and number of pigs born alive. Hopefully, the end result will be a relevant summary of their current effectiveness for improving fertility as well as an assessment of subsequent research needs so that the swine industry can realize their full potential for reproductive management of the breeding herd.

Boar seminal plasma proteins

Analytical techniques such as two-dimensional gel electrophoresis, gas and ion-exchange chromatography, western blotting, and mass spectrometry have allowed for extensive characterizations of the protein composition of porcine seminal plasma. A comprehensive review of all the putative proteins and polypeptides found in boar semen is beyond the scope of this paper and readers are referred to excellent articles on this topic that have been published elsewhere (Strzezek *et al*., 2005; Mogielnicka-Brzozowska and Kordan, 2011). Nevertheless, a brief synopsis of the wide array that is present seems appropriate. Most groups investigating the composition of seminal plasma use two-dimensional gel electrophoresis which causes proteins to migrate to a unique location based on their molecular weight (kDa) and isoelectric point or pH (Fig. 1). Depending on how samples were processed prior to electrophoresis and several other factors between 50 and 150 proteins typically migrate to unique locations (Strzezek *et al*.,

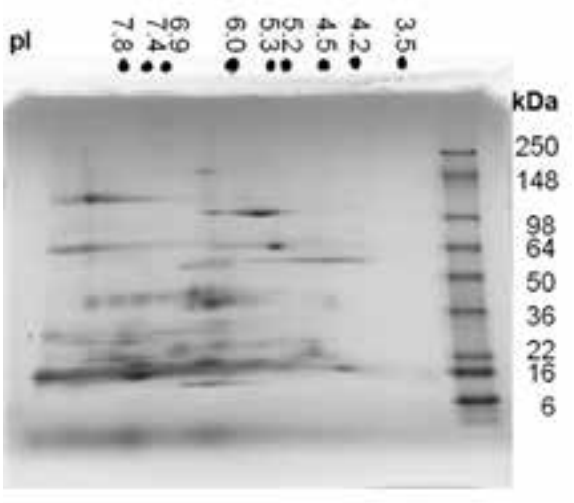

Fig. 1 Example of typical migration pattern of proteins from seminal plasma of boars subjected to two-dimensional gel electrophoresis. Molecular weight standards (kDa) are located in the right hand margin and isoelectric point standards (pH) are located along the top margin.

2005; Turner, 2008; Novak *et al*, 2010; Mogielnicka-Brzosowska *et al*., 2011). The majority of these have not been identified with additional techniques such as mass spectrometry, amino acid sequencing, or western blotting. Most of the proteins in seminal plasma have molecular weights between 12 and 22 kDa and a neutral to slightly basic pH, 7.0 – 8.4. This migration pattern is consistent with that of spermahesin proteins. Specific ones that have been identified in porcine semimal plasma include AQN-1, AQN-3, AWN-I, AWN-II, PSP-I and PSP-II (Kwok *et al*., 1993; Caballero *et al*., 2008). Collectively, they represent about 60% of the total proteins in porcine seminal plasma with the most abundant being a glycosylated heterodimer formed by the combination of PSP-I and PSP-II (Calvete *et al*., 1997). Other proteins not in the spermadhesin family, but present, albeit, in much smaller quantities include epididymal secretory glutathione peroxidase-5 (Jelezwsky *et al*., 2008; Novak *et al*., 2010); heat shock proteins (Turba *et al*., 2007); osteopontin (Novak *et al*., 2010); platelet-activating factor acetylhydrolase (Kordan *et al*., 2007); protein tyrosine acid phosphatase (Wysocki & Strzezek, 2003); poly-mannose glycoproteins (Strzezek *et al*., 2002); a sperm motility inhibiting factor (Kordan *et al*., 1998); tumor-necrosis factor α (Turba *et al*., 2007); transforming growth factor-β (O'Leary *et al*., 2011); and a family of zinc-binding proteins that can aggregate with each other in a number of different ways (Strzezek and Hopfer, 1987). It probably is safe to conclude that, from a qualitative perspective, only a small fraction of the proteins in seminal plasma that localize on 2-dimensional gels have been definitively identified.

Enhancement of porcine reproductive technologies with seminal plasma proteins

As mentioned previously, most of the reproductive technologies involving boar sperm require that seminal plasma be either significantly diluted or completely removed. A general concern with all of these techniques is that dilution or removal of the seminal plasma might impair the ability of sperm to successfully navigate the female reproductive tract and bind to ova, thereby compromising fertility. Technically, this is true for A.I. with fresh semen since ejaculates routinely are diluted 15 to 30-fold, but probably holds much greater relevance for frozen and sex-sorted sperm since each of these procedures typically use centrifugation or sedimentation to deliberately remove seminal plasma. During ejaculation spermahesin proteins attach to the heads of sperm, thereby, stabilizing the acrosome and preventing capacitation (Töpfer-Petersen

et al., 1998; Caballero *et al*., 2008). This is necessary because once capacitation begins sperm essentially have started down a path that will either allow them to fertilize ova or result in their death. Thus, spermahesin proteins in seminal plasma provide one mechanism by which sperm can remain viable in the female reproductive tract in an uncapacitated state until just prior to fertilization (Rodriquez-Martinez *et al*., 2005).

One of the major problems caused by extreme dilution of seminal plasma is premature capacitation. Capacitation-like changes have been well-documented in sperm subjected to cryopreservation (Rath *et al*., 2009) and sex-sorting protocols (Johnson *et al*., 2005) and often are blamed for their shortened lifespan and decreased fertility. This has stimulated research examining whether addition of whole seminal plasma or specific isolated proteins during various stages of these procedures can prevent premature capacitation and improve sperm quality.

Results from selected studies in this area are summarized in Table 1. These studies are similar in that the original protocol (control treatment) in each one, regardless of whether it was for cryopreservation or sex-sorting, initially required that sperm be separated from seminal plasma before being processed further. However, they differ in terms of the source of the seminal plasma and when during processing it was combined with sperm.

The general consensus from investigations with sex-sorted sperm is that inclusion of 10% seminal plasma during the staining (pre-sorting) and collection (post-sorting) phases has positive effects on sperm survival parameters and seems to attenuate capacitation-like changes. Unfortunately, it also appears that these improvements are at the expense of sperm's

Table 1. Summary of selected studies examining the effect of adding seminal plasma (SP) on quality of sperm subjected to cryopreservation or sex-sorting protocols.

Study	*Treatments*	*Timing*	*Results*
Sex-Sorted Sperm			
Maxwell *et al*., 1997 Maxwell *et al*., 1998	10% SP	During staining & pre-sorting	SP improved viability and membrane integrity & decreased capacitated sperm
Maxwell *et al*., 1997 Maxwell *et al*., 1998	10% SP	Post sorting & collection media	SP improved viability & membrane integrity & decreased capacitated sperm, but also decreased in vitro fertility
Garcia *et al*., 2007	10% SP	Post sorting	No effects on motility, membrane integrity, or in vivo ova penetration
Cryopreserved Sperm			
Hernández *et al*., 2007	5% SP from "good freezer" boars or 5% SP from "poor freezer" boars	After washing & before freezing	SP from "good freezers" improved post thaw motility and in vitro ova penetration
Saravia *et al*., 2009	SP 1 from first 10 mL of ejaculate or SP 2 from rest of ejaculate	After washing & before freezing	SP 1 improved post thaw motility
Vadnais & Roberts, 2010	10% SP + cooled to 5oC or 10% SP + cryopreserved	After washing & before freezing	10% SP prevented capacitation in cooled but not frozen sperm
Okazaki *et al*., 2012	15% SP or 50% SP	After washing & before freezing	No effects on post thaw motility
	15% SP	During thawing	SP decreased acrosomal cap loss, increased motility, and increased in vivo fertility

fertilizational potential in vitro. Effects of seminal plasma on sex-sorted sperm used to inseminate sows in vivo have not been reported. However, results from work conducted in sheep are not encouraging (Leahy *et al*., 2010). In this study, seminal plasma had no effect on fertility when it was added to sex-sorted, frozen-thawed ram sperm that were inseminated transcervically. This outcome has relevance for swine because this is the approach that would most likely be used to produce sex-sorted A.I. doses and then inseminate sows. Because seminal plasma is a mixture of many compounds, attention has turned to identification of specific proteins that are responsible for the observed positive effects on viability (de Graaf *et al*., 2008). Logical candidates are those in the spermahesin family. Unfortunately, addition of the PSP-I/PSP-II heterodimer to sperm after sorting did not have positive effects on motility, membrane integrity, or in vivo penetration of ova (Garcia *et al*., 2007), so clearly work in this area needs to continue.

In contrast, results from studies examining the effects of seminal plasma on the viability and subsequent fertility of frozen-thawed semen seem to be equivocal (Table 1). However, it appears that there is a reasonable explanation for this apparent ambivalence. The majority of the studies that have reported a positive effect have one of two things in common. They have used seminal plasma either from boars that have a history of high post-thaw quality or from fractions produced early during ejaculation whose sperm have been shown to have enhanced abilities to withstand cryopreservation. The finding that the addition of seminal plasma alone can significantly improve sperm characteristics of boars with a history of poor cryopreservation makes a strong argument for the involvement of specific proteins in preventing premature capacitation-like changes associated with freezing and thawing (Hernández *et al*., 2007). These observations were extended recently by Garcia and co-workers (2009) who demonstrated that, within the same boar, seminal plasma from the initial portion of the ejaculate can improve viability, motility and other characteristics of sperm recovered from subsequent fractions subjected to high dilution rates. This offers the intriguing possibility that all boars might possess these proteins in their seminal plasma, but the "good freezers" may simply have either a higher quantity of them in their early fractions or the volume of their early fractions represents a greater proportion of the total ejaculate compared with "poor freezers". If the proteins responsible for these beneficial effects can be identified and incorporated into cryopreservation and sex-sorting protocols, then, hopefully, the major obstacle of premature capacitation can be addressed.

Dilution of seminal plasma also occurs during the preparation of insemination doses with fresh semen. However, levels apparently are not severe enough to elicit the same effects on sperm as those observed after cryopreservation and sex-sorting because many swine operations achieve excellent fertility results with A.I. Nevertheless, there is good evidence that supplementation of A.I with seminal plasma may be able to enhance reproductive performance in certain situations. It is important to recognize that inclusion of seminal plasma to A.I. regimens using fresh semen probably is analogous to attempting to make a very good system reach peak efficiency, whereas adding it to cryopreservation or sex-sorting protocols is similar to trying to make a marginal system acceptable. Hence, the magnitude of the response is likely to be small in comparison.

Once introduced into the sow's reproductive tract seminal plasma can stimulate important changes thought to be important for a successful pregnancy. One of these is its ability to attenuate the inflammatory response associated with insemination. Introduction of semen into the female reproductive tract initiates a cascade of immune-related events that can have negative effects on uterine function and sperm survival (Robertson, 2007). Seminal plasma in semen, especially its protein component, significantly dampens these effects (Rozeboom *et al*., 1999) which, in turn, appears to be important for subsequent fertilization and implantation (Rozeboom *et al*., 2000). There is evidence that the PHP-I/PHP-II heterodimer contributes to this phenomenon, but there are likely other proteins as well that can stimulate similar responses (Rodriquez-

Martinez *et al*., 2009). Most of these appear to be in the cytokine family (Robertson, 2007). Regardless of which ones are involved, in theory, exposure of the sow's reproductive tract to seminal plasma prior to the entry of sperm should be an effective strategy for minimizing any negative consequences of insemination-induced inflammatory reactions. The general idea is that pre-treatment with seminal plasma would dampen the immune responses so that when mating occurred sperm would be introduced into a "quieter uterus", so to speak, in terms of its ability to initiate the cascade of events associated with inflammation.

A critical evaluation of several studies (Flowers and Esbenshade, 1993) in which the effectiveness of different combinations of pre- and post mating treatments with seminal plasma were evaluated provide support for this speculation (Fig. 2). Briefly, the original objective of these studies were to see if administration of seminal plasma pre- or post mating could be used to affect ovulation and essentially produce reproductive performance from single matings equivalent to that achieved with multiple inseminations. The standard insemination regimen on this farm was to breed sows naturally on the first day of estrus followed by A.I. on the second day. Data from the pre- and post mating treatments were from two different studies conducted on the same farm 6 months apart which is a less than ideal situation for comparison. However, each study had two identical treatments – natural service on day 1 followed by A.I. on day 2 and natural service only on day 1. Reproductive performance within each of these treatments between the two studies was similar which indicates that the overall fertility level in the herd did not change significantly from the beginning of the first study (pre-mating treatments) through the end of the second one (post mating treatments) and adds credence to the argument that

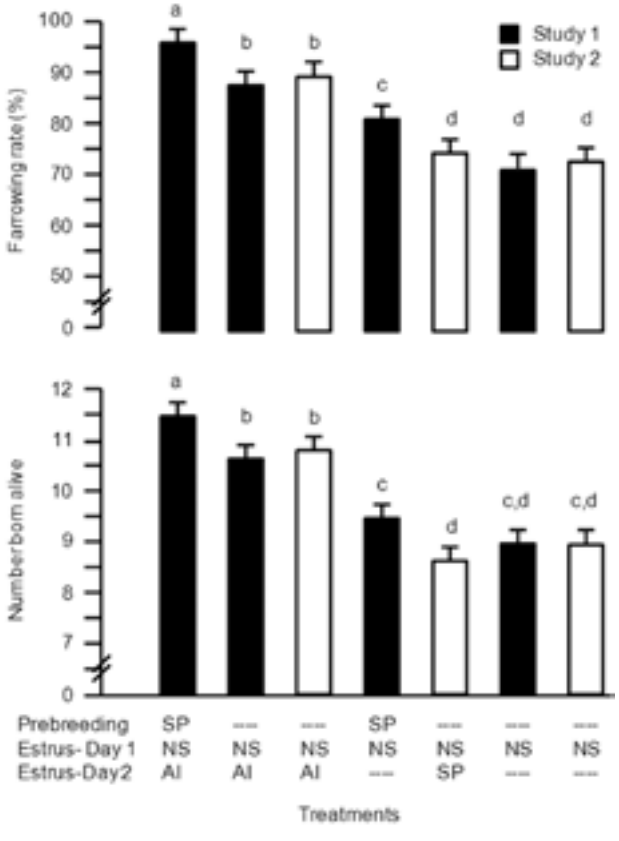

Fig. 2 Farrowing rate (top panel) and number of pigs born alive (lower panel) from sows given seminal plasma (SP) before or after being bred with various combinations of natural service (NS), artificial insemination (AI) or neither (----). Means with different superscripts differ significantly ($p \leq 0.05$).

observed differences across both studies are due to effects of seminal plasma administered pre- or post mating.

Administration of seminal plasma as a pre-mating stimulus produced exceptional fertility with farrowing rates and litter sizes greater than 95% and 11.5 piglets, respectively. It is important to recognize that these were statistically greater compared with standard mating regimens of a natural service on the first day of estrus followed by A.I. on the second day which resulted in very good performance – average farrowing rates and numbers born alive of 88.8% and 10.7, respectively. Results from the single mating treatments also illustrate the positive effects of seminal plasma. Mean responses in terms of farrowing rate and litter size on this farm to a single natural mating on the first day of estrus were 71.3% and 9.1 pigs, respectively. When sows were administered seminal plasma prior to the single natural mating these parameters increased by 10% and 0.5 pigs, respectively. Conversely, there was no effect on fertility when sows were treated with seminal plasma after being bred once naturally. These observations support the statement made earlier - inclusion of seminal plasma as a pre-breeding treatment has the potential to make very good performance better even if improvements are small quantitatively.

An alternative approach to using seminal plasma as a pre-mating treatment would be to increase the relative volume of seminal plasma in each insemination dose. However, this could have negative effects on sperm viability due to a reduction of the extender/semen ratio in the final storage volume (Johnson *et al*, 2000). Unfortunately, controlled studies directly comparing these two strategies are lacking. If the specific proteins in seminal plasma responsible for this effect can be identified, then enrichment of A.I. doses with them might prove to be a viable option. With recent advances in induced ovulation and intrauterine insemination protocols for sows, it is clear that sperm numbers and insemination volumes are likely to decrease which would also result in the exposure of the female reproductive tract to even less seminal plasma compared with conventional A.I. This raises the possibility that development of strategies to capture its positive effects on the female reproductive tract associated with dampening the immune system may become increasingly important for these advanced insemination techniques.

Seminal plasma proteins as markers for boar fertility

It is generally accepted that the commercial swine industry is doing a commendable job of screening for subfertile boars by monitoring their motility and morphology (Knox *et al*., 2008; Flowers, 2009). However, it also has been well documented that boars within the normal ranges for these parameters can differ considerably in their fertility (Flowers, 1997; Xu *et al*., 1998; Ruiz-Sanchez *et al*., 2006; Flowers, 2009). For example, some boars whose semen consistently has at least 85% normal motility and morphology produce low farrowing rates and litter sizes. Conversely, there are others with similar quality estimates that have exceptional fertility even when only 1 billion sperm are inseminated (Flowers, 2002). This has prompted interest in examining relationships between boar fertility and seminal components other than sperm. This approach has proved fruitful in beef and dairy cattle and led to the identification of several seminal plasma proteins that are highly correlated with male fertility (Killian *et al*., 1993). Consequently, this also appears logical to pursue for swine.

A critically important consideration associated with this line of research is determining how boar fertility should be measured. The relationship between sperm numbers and boar fertility resembles an asymptotic curve with a positive slope (Flowers, 2002). Initially, increasing sperm numbers increases fertility. This response gradually diminishes until a plateau is achieved above which additional increases in sperm numbers does not improve fertility. Consequently, the best chance of detecting fertility differences among individual boars is to inseminate numbers

of sperm associated with the linear portion of the curve and prior to the plateau. These are commonly referred to as suboptimal doses and generally contain between 1 to 2 billion sperm. Insemination doses associated with the plateau are less likely to reveal differences because some sperm traits are compensable. Compensable traits are those typically associated with the ability of sperm to bind to ova (Saacke *et al*., 2000; Braundmeier and Miller, 2001). Fertility of males whose sperm possess these traits can be improved simply by increasing the number of sperm inseminated which could mask differences at doses where the fertility curve reaches a plateau.

Results from studies investigating relationships between various seminal plasma proteins and boar fertility are summarized in Table 2. It is important to reiterate that in each of these studies, all the boars examined had motility and normal morphology parameters greater than 80%, so their semen would be used to make A.I. doses without hesitation in commercial boar studs. There also are a few important differences among these studies that should be considered when

Table 2. Summary of selected studies examining relationships between seminal plasma proteins and in vivo fertility in boars.

Study	*Seminal plasma proteins*[a]	*Correlation with in vivo fertility*[b]	*In vivo fertility range*[c]
Flowers, 1995*	25 kDa, pI 5.9	+ 0.45 – Farrowing rate + 0.49 – Total born + 0.47 – Fertility index[d]	75 – 95% 7.5 – 11.8 pigs 5.6 – 11.2
	55 kDa, pI 4.8	+ 0.56 – Farrowing rate + 0.58 – Total born + 0.58 – Fertility index	
Turner, 2008†	19 kDa, pI 6.8	- 0.21 – Piglets sired	8 – 88%
	25 kDa, pI 5.9	+ 0.71 – Piglets sired	
	55 kDa, pI 4.8	+ 0.68 – Piglets sired	
	Heat Shock Protein 70	+ 0.36 – Piglets sired	
Novak *et al*., 2010*	D-PSP-I	- 0.43 – Farrowing rate - 0.42 – Fertility index - 0.77 – Total born	71 – 98% 6.0 – 11.4 8.4 – 11.4
	Osteoponin-70	no significant correlations	
	AWN-I	no significant correlations	
	Glutathione peroxidase-5	+ 0.45 – Farrowing rate + 0.48 – Fertility index	
	60 kDa, pI 6.5	- 0.66 – Farrowing rate - 0.66 – Fertility index	

[a] molecular weights and isoelectic points (pI) were used for proteins that were not identified with additional techniques such as mass spectrometry or western blotting

[b] all identified proteins were included regardless of significance levels, but only unidentified ones that were either significant ($p < 0.05$) or exhibited significant tendencies ($p < 0.01$) were included.

[c] range in various fertility estimates for boars used in each study.

[d] fertility index is farrowing rate x total number born

* insemination of suboptimal numbers of sperm for estimates of boar fertility

† heterospermic inseminations and paternity testing for estimates of boar fertility

comparing results. In the study reported by Novak *et al*. (2010), ejaculates were collected in specific fractions in much the same way that was described for the cryopreservation and sex-sorting studies discussed previously. The initial sperm-rich fraction was the only one that was both analyzed for seminal plasma proteins and used to make insemination doses. In addition, the study was confined to 4 boars – the two with the highest and lowest in vivo fertility. In contrast, those conducted by Flowers (1995) and Turner (2008) collected, analyzed, and extended the entire ejaculate from 50 and 12 boars, respectively. Finally, the studies by Flowers (1995) and Novak *et al*. (2010) used suboptimal insemination doses of 1.5 billion sperm and conventional A.I. to breed sows and estimated boar fertility by evaluating the resulting farrowing rates and numbers of pigs born alive. In the study conducted by Turner (2008) heterospermic inseminations and subsequent paternity testing of the offspring were used. With this technique, 1 billion sperm from 3 boars were pooled together to make insemination doses and used to breed sows. Combinations of boars were organized so that all boars "competed" against each other. When piglets were born the paternity of each was determined and the relative fertility of each boar was assessed by determining the average number of piglets each sired. Each of the techniques used has its own set of strengths and weaknesses, but all are acceptable for detecting in vivo fertility differences among males.

A consistent finding among these studies was that a seminal plasma protein with a molecular weight around 25 kDa and a pI of 5.9 had a relatively high positive correlation with boar fertility. This protein was identified as glutathione peroxidase-5 by Novak *et al*. (2010). Glutathione peroxidases are key components of free radical scavenging systems (Drevet, 2006) and boar sperm are particularly sensitive to the effects of oxidative stress (Cerolini *et al*., 2000). Consequently, boars whose seminal plasma contains high levels of this protein presumably should be able to neutralize free radicals more effectively compared with their counterparts that do not. Thus, it is reasonable to speculate that they also should have increased fertility.

Given its migration pattern, it seems reasonable to assume that the 19 kDa, pI 6.8 protein in the study of Turner (2008) probably is a member of the spermahesin family, possibly PHP-I. Thus, negative correlations between fertility measures and D-PHP-I also seem to be fit nicely with our current understanding of boar fertility. Spermahesin proteins bind to sperm heads and stabilize acrosomal membranes. This is necessary during transit through the female reproductive tract. However, once the sperm reservoir is established, these surface proteins have to be removed in order for capacitation to proceed (Töpfer-Petersen *et al*., 1998). Presumably, if this doesn't occur, then fertilization is compromised. Thus, there could be a link between high levels of this protein in seminal plasma and reduced fertility. It is interesting to note that when seminal plasma (Maxwell *et al*., 1998) or the PSP-I/PSP-II heterodimer (Garcia *et al*., 2007) were added to sperm after sex-sorting in vivo penetration of oocytes was decreased, as well.

It should not be surprising that there are some proteins associated with fertility in each of these studies that were not confirmed in the others and indicates that there is considerable work yet to do in this area. As outlined previously, some of these differences are technical in nature and related to differences in the number of boars studied; procedures used to estimate in vivo fertility; and portions of the ejaculate from which seminal plasma was obtained. However, there also is a reasonable physiological explanation. This statement is based on lessons that have been learned from studying characteristics of fertile sperm (Braundmeier and Miller, 2001; Foxcroft *et al*., 2008). It is clear that sperm acquire their fertilizational competence via a series of well-coordinated events. If one is deficient, then no matter how potent the others may be, fertilization fails. As a result, the general consensus currently is that multivariate sperm traits such as ova penetration are better tests and yield more accurate estimates of sperm fertility compared with their counterparts that only quantify a single characteristic. This rationale

has relevance for use of seminal plasma proteins as indicators of boar fertility. It is clear that some seminal plasma proteins have direct effects on ejaculated sperm, while others stimulate important changes within the female reproductive tract during and after insemination. Thus, it is reasonable to speculate that there also is a coordinated sequence of events that seminal plasma proteins participate in and, as with sperm traits if one is minimal or is not present at all, then fertilization is compromised.

One way to address this possibility is to evaluate seminal plasma with an index derived from its complete protein composition as opposed to the presence of a single one. Proteins could be assigned a relative value based on a number of criteria including but not limited to the following: overall effect on fertility (positive or negative); strength of this relationship (correlation); and relative concentrations. Presumably, this would provide a more realistic evaluation of its impact on events related to fertilization and the establishment of pregnancy and, thus, of boar fertility compared with focusing on a single protein.

Preliminary results from a field study currently underway provide credence for this speculation (Table 3). This study is being conducted in a 50,000-sow commercial swine production system with two 250-head boar studs. Seminal plasma from selected boars (n = 25) is being analyzed quarterly for relative amounts of the 25 kDa, pI 5.9 and 55 kDa, pI 4.8 proteins (Flowers, 1995; Turner, 2008). Boars are ranked on the relative amounts of these proteins and insemination doses are made by pooling boars with high and low amounts of these proteins to form four basic treatments as shown in Table 3. At the present time, insemination doses made from boars with high levels of both proteins produce the best farrowing rates and litter sizes, while those with high levels of only one of these proteins exhibit a statistical tendency for better fertility compared with doses with low levels of both proteins. These initial results are encouraging and if the current differences are maintained, then development of fertility indices for boars based on the relative attributes of their complete seminal plasma protein profile is an area that should be pursued aggressively.

Table 3. Preliminary results investigating fertility of insemination doses produced from boars with divergent relative concentrations of 55 kDa, pI 4.8 and 25 kDa, pI 5.9 seminal plasma proteins (Flowers, W.L., unpublished)

	Treatments[a,b]			
Fertility estimates	*55 kDa – High* *25 kDa – High*	*55 kDa – High* *25 kDa – Low*	*55 kDa – Low* *25 kDa – High*	*55 kDa – Low* *25 kDa – Low*
Pregnancy rate, %[c]	96.3 ± 3.2* (121)	92.4 ± 3.1† (245)	91.6 ± 2.9† (221)	86.3 ± 2.5 (108)
Farrowing rate, %	93.2 ± 4.3* (113)	88.3 ± 3.8† (226)	87.7 ± 3.5 (202)	83.1 ± 3.3 (93)
Total born	12.3 ± 0.3* (105)	11.6 ± 0.3† (200)	11.7 ± 0.3† (177)	11.2 ± 0.3 (77)
Number born alive	11.6 ± 0.3* (105)	11.1 ± 0.3† (200)	11.3 ± 0.3† (177)	10.5 ± 0.3 (77)

[a] means ± s.e.
[b] numbers in parentheses indicate number of sows used to calculate each mean
[c] pregnancy rates based on real-time ultrasonography performed 28 to 34 days post breeding
*different from 55 kDa Low/25 kDa Low, $p \leq 0.05$
†different from 55 kDa Low/25 kDa Low, $p \leq 0.12$

Conclusions

There is considerable evidence that seminal plasma proteins hold potential for improving fertility associated with insemination of fresh, frozen, and sex-sorted semen as well as being able to accurately predict boar fertility prospectively. Those generally believed to be members of the spermahesin family have positive effects on sperm subjected to cryopreservation and sex-sorting. They improve viability, motility and acrosome intregity while reducing premature capacitation. Of particular interest are the observations that the ability of sperm to successfully withstand cryopreservation from some boars appears to reside, at least partially, in a specific fraction of seminal plasma and it is transferable to other individuals. Isolation and identification of putative proteins responsible for this phenomenon hold promise for improving fertility of sperm produced with these procedures which, in turn, would significantly increase their use by the swine industry. It is also clear that pre-breeding treatments with seminal plasma also hold potential for improving fertility on commercial swine farms that is already very good. The primary challenge with this application is to determine whether is a generic response to the total amount of protein inseminated or a specific one unique to individual proteins. If the latter proves to be true, then identification of these and subsequent enrichment of A.I. doses with them likely would be readily adopted. This may prove to be critically important as the popularity of induced-ovulation and transcervical insemination techniques increase. Finally, at least three seminal plasma proteins have been identified that consistently have reasonable correlations with in vivo fertility in a population of boars with excellent sperm motility and morphology characteristics that could be developed into prospective fertility tests. It is reasonable to speculate that as more proteins in seminal plasma are identified that this list will increase considerably. Nevertheless, it is important to recognize that as research in this area progresses a more holistic approach may be beneficial. Consideration of a boar's complete protein profile may prove to be more useful than focusing on a single one in terms of predicting his future reproductive potential.

Declaration of Interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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