Identifying useable semen
Swine Reproduction-Development Program, Swine Research & Technology Centre, University of Alberta, Canada

Abstract
The “predictors of useable semen” used in most commercial AI centers provide a very conservative estimate of the relative fertility of individual boars. Furthermore, the relatively high sperm numbers used in commercial AI practice (usually >3 x 10⁹ total sperm per dose of extended semen) usually compensate for reduced fertility, as can be demonstrated in some boars when lower numbers of sperm are used for AI. Differences in relative boar fertility are also masked by the widespread use of pooled semen for commercial AI in many countries. However, the need to continually improve the efficiency of pork production, suggests that commercial AI practice should involve increased use of boars with the highest genetic merit for important production traits. Necessarily, this must be linked to the use of fewer sperm per AI dose, fewer inseminations per sow bred, and hence more sows bred by these superior sires. In turn, this requires improved techniques for evaluating semen characteristics directly related to the fertilization process, such as IVM-IVF assays, analysis of seminal plasma protein markers, more discriminatory tests of sperm motility and morphology, with the goal of identifying high-index boars whose fertility is sustained when low numbers of sperm are used for AI. This paper reviews the current status of laboratory-based boar semen evaluation techniques that meet these criteria.

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1. Introduction
Overall reproductive efficiency of the swine herd is highly correlated to the reproductive capacity (fertility) of the males. Poor quality boars, because of the polygamous structure of swine production, will affect the reproductive outcome of numerous females. In the case of AI, this could be thousands of females. It is clear that not all boars and not all ejaculates from those boars are created equal; however, ejaculates collected for use in AI are subjected to a minimum standard of semen analysis. Using sub-fertile boars and low quality ejaculates reduces production efficiency and lowers profit margins for the producer.

The ultimate measures of boar fertility in production records are pregnancy rate and litter size born. However, these are retrospective measures of boar fertility and can be highly influenced by breeding management and quality of the females [1]. Livestock production managers have come to accept that a combination of a thorough physical examination of the boar and conventional semen evaluation (concentration, morphology, motility) can provide an alternative to actual fertility data [2]. Although these evaluations can establish that an animal is either sub-fertile or infertile, they cannot identify the relative fertility
of boars that meet accepted industry standards for sperm and ejaculate quality [3]. The “predictors of useable semen” currently used in most commercial AI centers provide a very conservative estimate of the relative fertility of individual boars. Furthermore, the relatively high sperm numbers used in commercial AI practice (usually $3 \times 10^9$ total sperm per dose of extended semen), and the pooling of semen from boars that may have inherently different fertility, likely mask the reduced fertility that can be demonstrated in some of these boars when lower numbers of sperm are used for AI, or if they are used on an individual basis.

If the full economic impact of the highest genetically indexed boars is to be realized at all levels of the breeding pyramid, then the number of gilts and sows bred per boar must be maximized. A number of innovations in insemination technology, including post-cervical [4] and deep-uterine [5] insemination are conducive to the use of lower sperm numbers per insemination. The further possibility of using controlled ovulation techniques to achieve single fixed-time insemination protocols [6] would also substantially increase the utilization of genetically superior boars. Therefore, effective predictors of relative boar fertility would be essential to exclude less fertile boars from commercial stud barns and thus optimize the use of proven high fertility, and genetically high-indexed boars with lower sperm numbers per AI dose. At the nucleus level, this will allow for increased selection pressure by increasing the number of offspring bred per collection from high-ranking boars. At the level of terminal line production, this would allow considerable improvements in production efficiency, by capitalizing on boars with a high-index for traits such as growth rate, feed conversion efficiency, and carcass characteristics of their progeny. Even if the same production costs were paid in genetic royalties, by purchasing fewer total doses of semen but from genetically superior boars, the cost benefits realized by producers in the grow-finish performance of the progeny produced and the quality of the carcass sold, would nevertheless be positive.

If the changes in production strategy described above are to be realized, it is critical to be able to identify boars of relatively low fertility when used in the more challenging situation of reduced sperm numbers per AI dose or per insemination. Thus, the very definition of “useable semen” changes in this new context. Existing information and recent research directed to achieving these more demanding criteria of useable semen objectives is presented below.

### 2. General approaches to boar semen evaluation

Researchers have searched for decades to find a single test or combination of tests that can accurately predict male fertility from a semen sample [7]. Unfortunately, there appears to be no simple answer to this very complex question [8]. Often laboratory assays examine all of the sperm present in a sample for fertility, yet only a few sperm are actually necessary to fertilize all available oocytes. Braundmeier and Miller [9] suggested that the sperm that fertilize the oocytes in vivo may be a small, highly selected, sub-population that is not representative of the average sperm evaluated in the sample. They also suggest that, because sperm must meet many requirements for successful fertilization, testing a single attribute is unlikely to be a true measure of ultimate fertility. Further, Rodriguez-Martinez [8] suggested that to accurately predict semen fertility, it is necessary to test all sperm attributes relevant for fertilization and embryo development within large sperm populations, and to develop in vitro techniques that will predict the fertility of low sperm doses used for AI. The author also suggests that these techniques must accommodate, and accurately predict, in vivo fertility.

Braundmeier and Miller [9] reviewed a number of functional and molecular tests used to assess male fertility. In this review, they describe two sperm traits that affect fertility. Compensable traits are those that can be overcome by introducing large numbers of sperm during insemination. Problems with motility and morphology will reduce the number of sperm that are able to reach the oocyte, but by introducing large numbers of sperm, the reduction in fertility can be minimized. Uncompensable traits are those that cannot be overcome by introducing larger numbers of sperm. These defects affect fertilization and embryo development and include nuclear vacuoles, sperm chromatin structure and morphological problems that do not inhibit movement. Therefore, to effectively predict fertility, it is essential to discriminate between compensable and uncompensable traits in an ejaculate.

Conventional semen evaluation generally includes a measure of semen volume, sperm concentration, and the percentage of sperm that are progressively motile and morphologically normal [10,11]. Although some of these parameters are correlated with fertility in the boar [12,13], several authors suggest that this information, while important, does not accurately predict whether a male is truly fertile [14–16]. As shown in Table 1, existing analyses are also generally inadequate for predicting relative fertility in healthy
boars with ejaculate quality that meets typical industry standards (>70% motility and <30% abnormal sperm) [12,17], even though the reproductive efficiency of these boars may still be substantially different [12,18–20]. As illustrated in Tables 2 and 3, differences in relative fertility become increasingly evident when low sperm doses (<2.5 × 10⁹ sperm) are used for AI [4,18,21,20]. This approach likely avoids the compensatory effect of using excessive sperm numbers per AI dose [17,22], allowing the detection of fertility differences among relatively fertile boars.

3. Semen evaluation considered in the context of the natural fertilization process

In reality, fertilization of an oocyte is a complex process requiring that a number of critical conditions and events take place in both the male and female. Preliminary steps in the preparation of sperm for ejaculation include sperm formation, sperm transport, and the addition to the ejaculate of seminal plasma components from the male accessory glands. Subsequently, within the female reproductive tract, several sperm attributes are required for successful fertilization. These include the ability of sperm to undergo capacitation, hyperactivation and the acrosome reaction, to bind to the zona pellucida (ZP) and penetrate the oocyte. Consequently, more discriminatory and sophisticated in vitro tests have been developed for evaluating semen characteristics directly related to the fertilization process. Furthermore, as these characteristics have a multiplicative relationship, even if one of these is compromised, the likelihood of successful fertilization drops dramatically. Therefore, it is imperative to evaluate as many of these factors as possible to

<table>
<thead>
<tr>
<th>Motility (%)a</th>
<th>Sperm penetration rate (%)b</th>
<th>Farrowing rate (%)c</th>
<th>No. born alive</th>
</tr>
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<tbody>
<tr>
<td>94.7</td>
<td>89.5 (58)</td>
<td>86.9 (460)</td>
<td>10.6</td>
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<tr>
<td>82.3</td>
<td>81.7vw (55)</td>
<td>87.1v (330)</td>
<td>10.5</td>
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<tr>
<td>76.1</td>
<td>84.3vw (50)</td>
<td>84.5v (300)</td>
<td>10.5</td>
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<tr>
<td>66.2</td>
<td>74.7w (44)</td>
<td>86.1v (264)</td>
<td>10.1v</td>
</tr>
<tr>
<td>52.4</td>
<td>55.5x (40)</td>
<td>72.4w (201)</td>
<td>9.2w</td>
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<tr>
<td>44.2</td>
<td>34.7w (28)</td>
<td>72.3w (168)</td>
<td>9.2w</td>
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<tr>
<td>32.6</td>
<td>21.3x (17)</td>
<td>51.7w (85)</td>
<td>7.8w</td>
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<tr>
<td>S.E.M.</td>
<td>4.8</td>
<td>5.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

v–zWithin a column, means with different superscripts differ (P < 0.05).
a Motility is expressed as the average percentage of motile spermatozoa within the following classes: >90; 80–89; 70–79; 60–69; 50–59; 40–49; and 30–39%.
b Sperm penetration rate is defined as the percentage of eggs that were fertilized. The numbers in parentheses represent the number of ejaculates within a motility category.
c Number in parentheses represent the number of sows inseminated within a motility category.

<table>
<thead>
<tr>
<th>Boar</th>
<th>No. gilts</th>
<th>Fertility in vivo</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bred</td>
<td>Pregnant</td>
</tr>
<tr>
<td>R-2</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>Y-2</td>
<td>53</td>
<td>48</td>
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<tr>
<td>Pu-3</td>
<td>57</td>
<td>54</td>
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<tr>
<td>B-1</td>
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<td>54</td>
</tr>
<tr>
<td>R-3</td>
<td>55</td>
<td>52</td>
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<tr>
<td>G-2</td>
<td>45</td>
<td>42</td>
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<tr>
<td>B-3</td>
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<td>51</td>
</tr>
<tr>
<td>R-1</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>G-1</td>
<td>51</td>
<td>37</td>
</tr>
</tbody>
</table>

P = 0.0003, P = 0.0003, P < 0.001, P < 0.0001

x–zMeans with different superscripts within each column were different (P < 0.05). a–dLSM with different superscripts within each column were different (P < 0.05). Values in the table are least-square means (LSM) ± standard errors of the mean (S.E.M.). P: probability of main effect of boar.
determine if a boar ejaculate is fertile and, therefore, capable of fertilizing a mature oocyte. Although certain semen characteristics have been successfully used to identify fertile vs. sub-fertile boars and/or ejaculates, it is unclear whether these techniques would be effective for predicting the relative fertility of boars with ejaculates that meet normal laboratory criteria for AI use.

4. Techniques for semen evaluation

Semen has several characteristics that can be evaluated by macroscopic, microscopic and functional analysis. Routine evaluations used in commercial boar studs may include color, odor, sperm numbers and ejaculate volume, ejaculate pH, and sperm motility, morphology and viability. These traditional measures of ejaculate quality verify the suitability of the ejaculate for extension and the number of doses per ejaculate that can be produced. On an ongoing basis, these characteristics also provide a measure of sperm production and the health of the boar. However, if more discriminatory tests of relative boar fertility are to be developed, those characteristics of the ejaculate that might contribute to such discriminatory analyses are of particular interest. These characteristics are summarized below and discussed in the context of potential discriminatory tests of relative boar fertility. Where relevant, evaluations that provide additional information on sperm that might be optimal for in vitro fertilization procedures are also discussed.

4.1. Sperm motility

Sperm motility and hyperactivation are important characteristics used to assess the sperm’s potential to move through the female genital tract and penetrate the oocyte. Sperm motility is an indirect measurement of sperm viability and can be assessed by subjective observation of sperm viewed under a microscope or by more objective measurements using Computer Assisted Semen Analysis (CASA), Mariker Counting Chamber, videotape recording, or photographic systems.

Flowers [12] concluded that the percentage of motile sperm only gives a qualitative estimate of fertility and is only useful for estimating the reproductive performance of boar semen with less than 60% motile spermatozoa (Table 1). Xu et al. [13] found no correlation between sperm motility and litter size, using 3 and 2 × 10⁹ sperm per AI dose to breed weaned sows with an early return to estrus. However, Tardif et al. [18] reported that when 0.3 × 10⁹ sperm were used per AI dose, the percentage of sperm with normal motility was highly and positively correlated with farrowing rate. Perhaps the use of large numbers of sperm per AI dose masks actual boar fertility potential, and therefore, sperm motility is a compensable trait.

In contrast to motility estimates in raw or extended semen on the day of collection, sperm motility in extended semen at day 7 has been correlated with in vitro fertility estimates [23], whereas reported correlations with in vivo fertility are variable [13,24,25]. Subsequently, Ruiz-Sanchez et al. [3] established significant correlations between the motility of extended semen on days 7 and 10 and farrowing rate and total litter size born in groups of gilts bred with 1.5 × 10⁹ sperm per AI dose. Therefore, the motility of extended semen at different days of storage may offer a practical and inexpensive approach to identifying relative boar fertility. However, more studies are needed to confirm this relationship and
should involve more precise methods of measuring sperm motility and motility characteristics.

4.2. Sperm viability

Vital stains and the hypo-osmotic swelling test (HOST) are used to estimate sperm viability. Comparisons have shown that HOST results had weak or no correlation with the vital stain results (Eosin-nigrosin, Trypan Blue, IP-CFDA, and IP-SYBR). These differences are likely due to the fact that sperm characteristics measured by HOST are quite different from the vital stains. In that regard, HOST evaluates the sperm membrane’s ability to regulate the flux of electrolytes and non-electrolytes, and sperm plasma membrane integrity, whereas vital stains simply evaluate membrane integrity and not its biochemical activity [26–28].

Several studies have demonstrated that sperm viability results obtained with vital stains or fluorescent vital stains have no significant correlation with fertility in vitro [28,9] or in vivo [30]. Nevertheless, there is evidence for positive correlations between the HOST, and zona pellucida penetration [31] and in vitro fertilization rates [28,32]. Unfortunately, HOST results have negligible or no correlation with in vivo fertility in boars [33].

4.3. Sperm morphology

Sperm morphology is evaluated using various techniques and stains, such as Eosin-nigrosin, Trypan Blue, Giemsa, Papanicolaou, and Diff-Quik [34]. Since some of these stains can be imprecise, an alternative method is to fix the sperm sample with formalin or formol citrate, and analyze the sample with phase-contrast microscopy. Other techniques used for assessing sperm morphology include the use of transmission electron microscopy and CASA.

Berger and Parker [35] did not find any correlation between sperm morphology and boar fertility in a competitive mating situation. However, Xu et al. [13] found that morphological characteristics are a useful tool for assessing semen quality in boars, in that differences in normal sperm morphology contributed to the variance in litter size born when 2 \times 10^9 normal and motile sperm per AI dose were used for AI in weaned sows.

4.4. Sperm DNA evaluation

In recent years, sperm DNA structure has been evaluated as a cause of male infertility. Damage to spermatozoa DNA may occur at different points in the male reproductive tract (testis or epididymis), as well as during semen collection, semen processing (semen extension for freezing) and semen storage, and could have a negative impact on fertilization and fetal development [36–39]. Techniques used to evaluate DNA and chromatin quality include: Comet, transferase mediated dUTP nick-end labeling (TUNEL), sperm chromatin structure assay (SCSA), and the sperm chromatin dispersion test. The main objective of these tests is to evaluate the degree of DNA fragmentation in semen samples in order to assess semen quality.

Strong positive correlations have been found between DNA fragmentation index (DFI) in boar semen and routine semen evaluation parameters such as motility (%), normal acrosomes (%), abnormal sperm (%), and cells positive to HOST [37]. Differences in sperm DNA Fragmentation Index have been found between different boar breeds [39] and between individuals of the same breed [37]; therefore, DNA evaluation could be used as an additional test for assessing semen quality and boar fertility.

5. Integrated evaluation of sperm function

Several investigators have developed tests that are useful for evaluating sperm functions that are more directly related to the fertilization process, such as the ability to undergo capacitation, hyperactivation, the acrosome reaction, sperm membrane fusion, zona pellucida penetration, and fertilization of the oocyte.

5.1. Sperm–zona pellucida binding test

Sperm binding to the zona pellucida has been assessed using hemizona (HZA) and zona pellucida binding assays, which measure the ability of sperm to approach and attach to the homologous zona pellucida of immature eggs (species specific). Low sperm binding to the zona pellucida may indicate issues related to the capacitation process [40], but associations with male fertility have been variable. For example, a sperm–zona binding assay was used with cryopreserved ram and fresh boar semen [29] and in neither species was zona binding correlated with in vivo fertility. Interestingly, Fazeli et al. [41] used the zona pellucida binding test to show that a high percentage of the sperm that initiate binding to the zona pellucida have an intact acrosome. This may be a clear indicator of why low fertility rates are obtained with cryopreserved semen. Perhaps the sperm–zona pellucida binding test could be used to
predict in vitro fertilization capacity and as a complementary test for assessing in vivo fertility.

5.2. Oocyte penetration assay

The classic sperm penetration test uses zona-free hamster eggs in an in vitro assessment of sperm functional competence to fuse with the egg membrane, and to undergo decondensation. Several studies have reported a high correlation between penetration rate (zona-free hamster ova assay) and male fertility. For example, sperm from fertile boars had a high penetration rate compared with sperm of infertile boars [35,42,43]. Subsequently, Martinez et al. [44] used zona-free pig oocytes (a homologous in vitro penetration assay (hIVP)) to study the relationship between the hIVP and boar fertility, and concluded that this assay effectively identified fertile from sub-fertile boars. Despite encouraging results, in all these studies, the ability to identify differences in relative fertility among fertile boars was limited.

5.3. In vitro fertilization assay

In vitro fertilization (IVF) may be used to assess the ability of sperm to fertilize a homologous zona-intact egg, generally using immature oocytes collected from fresh ovaries of prepubertal gilts and matured in vitro, a process termed oocyte in vitro maturation (IVM). Immature frozen oocytes (germinal vesicle stage) have also been used to reduce the cost and time involved in the oocyte maturation process and to reduce the variation in oocyte quality between replicates of IVF within the same experiment [44]. Parameters evaluated include penetration rate, polyspermy rate, monospermy rate, male pronuclear formation rate (MPN), number of sperm per oocyte, and potential embryo production rate [13]. This test evaluates the spermatozoa’s potential to complete several changes (biochemical and biophysical) such as capacitation, the acrosome reaction, sperm–zona binding, sperm–oocyte binding and penetration, and sperm decondensation, which are all required for successful fertilization.

Based on the IVF data obtained, in vitro characteristics that are not affected over time but differ among boars are potentially useful as predictors of fertility in vivo. In the studies of Ruiz-Sanchez et al. [3], male pronuclear formation rate was the only IVF variable that explained some of the variation in fertility in vivo (from 12 to 17%). Overall, other IVF characteristics lacked strong correlations with in vivo fertility, suggesting that thresholds for sperm quality were being met when relatively fertile boars were compared. However, sperm from the lower fertility boars in this study produced lower oocyte penetration and MPN-formation rates and critical thresholds for such traits (e.g., >50% oocyte penetration rate) can perhaps be used to identify sub-fertile boars.

The processes involved in IVF compared to in vivo fertilization may also contribute to the low correlations between in vivo and IVF data. Existing IVM and IVF systems have often been optimized for assessing oocyte quality and embryo production potential [45–53] and there is a need to improve IVM/IVF techniques for the specific purpose of sperm evaluation. The use of standardized total sperm numbers per oocyte for IVF, without any adjustments for motility after sperm capacitation, in vitro, would probably help to identify the variation in sperm quality between the boars that affect the efficiency of fertilization process. Another approach could be to use much lower numbers of sperm per oocyte for IVF, thus placing the sperm in similar challenging situations in vitro and in vivo. Likewise, the use of the same ejaculate fractions for both in vivo and in vitro fertility evaluations could confirm the relationship between them. Recent studies by Rodriguez-Martinez et al. [54] demonstrated that the sperm from the sperm peak (SP) fraction of the ejaculate are superior (sperm membrane integrity, percentage of live cells, etc.) than sperm from the bulk ejaculate (including subsequent Sperm-Rich fractions and Sperm-Free fractions of the ejaculate). These results may provide a better understanding of why the SP fraction presents the least variability when used for in vitro fertilization [55], and represents the best sperm sub-population to test in order to obtain a predictor of fertility. The difference between these fractions is likely produced by interactions with seminal plasma components that may not influence the sub-population of sperm that rapidly establish themselves in the oviductal reservoir in vivo [54,56]. Therefore, further investigation needs to be done in this area to better understand sperm and seminal plasma interactions in vivo.

From a practical perspective, even if certain IVF parameters are correlated with in vivo boar fertility, such as oocyte penetration rates and number of sperm attached per oocyte, male pronuclear formation rate, and potential embryo production [13], this technique is expensive and time consuming. It is, therefore, unlikely to be practical for routine semen evaluation at commercial level. However, if the results of such tests are predictive of relative life-time fertility, and can be carried out during the initial quarantine and training period of potential stud boars, then these tests
may still make an important contribution to boar fertility assessment.

5.4. Sperm–oviduct binding assay

Recently, the in vitro evaluation of the sperm’s capacity to bind to the oviductal epithelium or oviductal cells has been used to understand sperm–oviductal interactions [57–62], as well as to detect differences in boar fertility [63,64]. Differences in the binding index between boars of different fertility indicate that this test could be useful as a complementary test to assess boar fertility and gives potentially valuable information on any changes in sperm binding function during storage [65]. It is also important to note that the sperm population in the oviductal reservoir depends on the initial sperm quality (ejaculate and/or boar), the site of sperm deposition, number of sperm inseminated, and may, therefore, reflect differences in ejaculates and male fertility.

6. Seminal plasma proteins and male fertility

As seminal plasma proteins play an important role in the fertilization process, several studies have investigated the action of those proteins before and during fertilization. Flowers [12] demonstrated that mixing seminal plasma of high fertility (“dominant”) boars with the sperm of low fertility (“non-dominant”) boars improved the fertility of the sperm used for in vivo fertilization. Furthermore, there was a reduction in fertility when sperm of dominant boars was exposed to seminal plasma of non-dominant boars. Likewise, Zhu et al. [56] found differences in oocyte penetration rates in vitro when spermatozoa from the SP fraction were pre-incubated with various fractions of seminal plasma from the same ejaculate. Subsequently, Caballero et al. [66] reported negative effects of seminal plasma from specific boars on viability of sperm after extension. Finally, beneficial effects of including seminal plasma during different stages of the sperm cryopreservation process [67–71] and when cryopreserved sperm are thawed and used for AI [72,73] have been demonstrated. Collectively, this information demonstrated the important role of seminal plasma, and particularly seminal proteins, during the fertilization process. Therefore, it would still likely be beneficial to identify fertility markers present in seminal plasma and include these as part of the assessment of ejaculate quality and boar fertility.

Important physiological functions of seminal plasma have been described. Firstly, it transports spermatozoa from the male tract into the female genital tract. Spermatozoa are produced in the testes and then matured and stored in the epididymis. During ejaculation, the sperm are mixed with epididymal secretions, transported from the cauda epididymis through the deferent ducts to the urethra, where they are mixed with the secretions of the accessory sex glands before finally being ejaculated (into the female tract) by muscular contractions of the urethra. Seminal plasma also acts as a buffer solution and is a source of nutrients, providing the energy substrates (glucose, fructose, etc.) that increase the life span of the spermatozoa. Binding of specific seminal plasma proteins to the sperm plasma membrane is involved in sperm–oviductal binding, prevention of early capacitation, maintenance of sperm viability, sperm–zona pellucida interactions, and oocyte–sperm binding. At the uterine level, specific seminal plasma proteins seem to control the neutrophil response. There is also evidence that seminal plasma components play an active role in the female reproductive tract, enhancing uterine contractions, advancing the time of ovulation, and eliminating non-viable spermatozoa. However, from the perspective of using analysis of seminal plasma to characterize relative boar fertility, it is important to recognize that seminal plasma composition and volume differ among species, individuals, ejaculates from the same individual, and even among various fractions of individual ejaculates. Nevertheless, an understanding of the roles of seminal plasma in the complex peri-fertilization events in mammals helps to assess the potential for using seminal plasma components as indicators of relative fertility. The functions of seminal plasma components in the boar are, therefore, presented below with the functionality of seminal plasma proteins being classified as: (1) spermadhesins, (2) sperm motility inhibitory proteins, and (3) decapacitation factors.

The spermadhesins identified in boars are AWN, AQN-1, AQN-3, PSP-I, and PSP-II, as well as the glycosylated isoforms of AWN, AQN-3, PSPI, and PSPII (monomers and dimers). These proteins coat the sperm plasma membrane during their transit through the epididymis and during ejaculation, producing structural changes to the sperm surface. In general, they are considered to maintain plasma membrane stability, play important roles in the sperm–oviduct interactions [63,74,75], capacitation, spermatozoa–zona binding [76,77] and sperm–oocyte interactions [77,78]. However, based on the low amounts of AWN attached to the surface of live ejaculated spermatozoa [79], it is probably not primarily responsible for the sperm–oviductal interaction during sperm reservoir formation.
AQN-1 and AQN-3 bind to the α- and β-galactose and α-mannose structures that have been related with the oviductal epithelium and zona pellucida binding, respectively [80,81], supporting the hypothesis that AQN-1 plays an important role in sperm–oviductal interactions and sperm reservoir formation.

Porcine Seminal Plasma protein (PSP) was identified by Rutherfurd et al. [82] as two subunits, PSP-I and PSP-II, with 80% homology. Both, PSP-I and PSP-II are N-glycosylated proteins composed of 110 and 116 amino acids [82,83] with a 12–14 kDa molecular weight, respectively [82,84]. PSP-I may prevent premature capacitation and acrosome reaction [77,85], and have immune-regulatory activity in the uterine environment [86–88]. PSP-II exhibits the capacity to bind to the glycoproteins of the zona pellucida, has affinity for soybean trypsin inhibitors, and could be involved in initial sperm–oocyte recognition and the acrosome reaction [89,90]. PSP-I/PSP-II heterodimers have also been reported, which bind weakly to zona pellucida glycoproteins 90 and are involved in a pro-inflammatory response [87,88].

Decapacitation factors present in the seminal plasma are generally proteins of high molecular weight that coat the sperm plasma membrane during ejaculation to prevent premature capacitation [91,92]. Thus, decapacitation factors present in seminal plasma bind to sperm surface during ejaculation, providing temporary protection from premature capacitation and the acrosome reaction while the sperm traverse the female tract.

Sperm motility inhibitor was first identified in boar seminal plasma by Iwamoto et al. [93] as a 50-kDa protein, and subsequently identified as an aggregation of three polypeptides of 12–18 kDa that exhibited close similarity to the AQN-3 spermadhesin [94,95]. Therefore, the binding of associated AQN-3 proteins to the sperm membrane produces sperm motility inhibition. Sperm motility is thought to be re-established when these molecules are removed from the sperm surface during capacitation.

6.1. Seminal plasma proteins as markers of boar fertility and semen quality

Specific seminal plasma proteins have been identified as potential markers of male fertility in the bull [96], stallion [97], and boar [98]. Flowers [98] working with ejaculates associated with a large range in fertility (86.7 ± 3.4 to 62.7 ± 4.0% farrowing rates and 11.2 ± 0.3 to 8.6 ± 0.4 average born alive, respectively) was able to identify two seminal plasma proteins (55 kDa, pI 4.5 and 26 kDa, pI 6.2) that were positively associated with in vitro and in vivo fertility. More recently, Ruiz-Sanchez [20] reported that fertility in vivo was negatively correlated (Figs. 1 and 2) with relative abundance of PSP-I, and with both a 20 kDa, pI 6.0 and 60 kDa, pI 6.5 protein, but positively correlated with a 25 kDa, pI 5.9 protein, which could be homologous to a fertility associated seminal plasma...
protein reported in the boar [98], bull [96], and stallion [97,99]. The specific role of the lipocalin-type prostaglandin D synthase protein identified in the stallion is still not confirmed; however, Gerena et al. [99] suggested a role in sperm development and maturation, due to its presence on the apical ridge of the sperm acrosome. The identification of this protein in the boar and its role during the fertility process require further investigation. Furthermore, Ruiz-Sanchez [20] also identified a 70 kDa protein as osteopontin, which has also been associated with differences in bull [100,101] and stallion fertility [97]. Collectively, these results confirmed that specific seminal plasma proteins are indeed associated with boar fertility, providing the basis for their use as a complementary tool to identify sires with high and low fertility potential.

Differences among various fractions of the ejaculate in the amount of seminal plasma proteins were also evaluated [20]. The Sperm-Peak fraction possessed lower total protein concentrations, as well as lower concentrations of specific seminal plasma proteins (PSP-I, AWN-1 and OPN), than the Sperm-Free fraction. This effect could be produced by a combination of factors: (1) seminal plasma origin, (2) sperm quality (sperm ability to absorb seminal plasma proteins), and (3) sperm concentration. These factors may, individually or collectively, determine the variation in total protein concentration among the seminal plasma fractions. Total seminal plasma protein concentration was negatively correlated with both zona pellucida penetration rate and the number of sperm penetrating the zona in vitro. This, along with differences in specific seminal plasma proteins (spermadhesins and decapacitation factors) between Sperm-Peak and Sperm-Free fractions, could explain the reduction in penetration rates observed by Zhu et al. [56], when sperm from the Sperm-Peak fraction were pre-incubated with seminal plasma from the Sperm-Free fraction. In a pilot study [20], differences in IVF parameters between ejaculate fractions (Sperm-Rich vs. Sperm-Peak) were evaluated. Although most of the characteristics associated with higher fertility were superior for the Sperm-Peak fraction, only average number of sperm penetrating the zona pellucida, and average number of sperm penetrating the oocyte, were significantly increased. In contrast, in an in vitro study, the inclusion of seminal plasma in sperm samples sorted by flow-cytometry increased the percentage of uncapacitated, acrosome-intact sperm, and reduced oocyte penetration rate [102]. Furthermore, in recent studies, exposure to PSP-I/PSP-II heterodimers maintained sperm motility, viability, and mitochondrial activity in highly extended ejaculates [66,103,104]. Therefore, we inferred that seminal plasma proteins have a predominantly decapacitating effect, reducing sperm penetration in vitro.

7. Conclusions

The ability to differentiate relative fertility amongst boars would have a substantial economic impact on the swine industry, by eliminating or optimizing the use of less fertile boars. It would also allow for the use of lower numbers of sperm from the most fertile and genetically
high-indexed boars per AI dose, without any loss in productivity. Evaluation of the motility of extended stored semen appears to provide a practical and inexpensive approach to identifying less fertile boars at an early stage. However, evaluation of this technique is required at a commercial level to confirm these relationships.

In contrast to more conservative evaluations of semen quality, the use of IVM/IVF assays demonstrates that this technique is able to discriminate between relative boar fertility and their likely productivity under more stringent (lower sperm numbers per dose) or demanding (use of ageing stored semen) conditions of AI use. However, further studies are again needed to optimize the sensitivity and repeatability of this and other in vitro techniques. Regardless, the complexity of these tests requires that they provide meaningful estimates of expected life-time performance of boars at an early stage of the boar evaluation process. Alternatively, the use of low sperm numbers per AI dose to breed gilts at their pubertal estrus, combined with the use of breed and abort protocols to determine the number of conceptuses at approximately day 30 of gestation, can also provide meaningful information on relative boar fertility at a reasonable cost. This program would have little impact on breeding herd productivity, but could produce invaluable data regarding the potential fertility of boars being considered for use in AI studs, and requires virtually no laboratory facilities.

Finally, evidence for a role of specific boar seminal plasma proteins in the fertilization process suggests that they may contribute to observed differences in semen quality and boar fertility. Further assessment of these potential fertility markers may provide the basis for using seminal plasma proteins to identify sires with high and low fertility.

References


