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Theriogenology
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Theriogenology 63 (2005) 431–444
www.journals.elsevierhealth.com/periodicals/the

Sperm factors related to in vitro and in vivo porcine fertility

Joaquín Gadea*

Department of Physiology, Faculty of Veterinary Medicine, Murcia University, Murcia, Spain

Abstract

The prediction of sperm fertilizing ability has great economic importance for breeding herds when artificial insemination is used. Classical methods of semen evaluation generally measure the sperm concentration, progressive motility, percentage of viable cells, and acrosome morphology. These assays are poor in predicting sperm fertility, because only the samples with markedly poor quality can be detected. The development of new sperm tests that measure certain sperm functions is an attempt to solve this problem. On the other hand, the binding and penetration of the zona pellucida is one of the most important barriers the spermatozoa must overcome in the fertilization process. Also, the interaction with the oocyte plasma membrane appears to explain much of the variability in sperm fertilizing potential among fertile boars. Thus, the study of the relationship between sperm factors and in vitro fertility may be a good strategy and assays that include a study of gamete interaction may lead to a better way to predict male fertility than the routine laboratory evaluation of semen.

This review will discuss the relationships between sperm factors and fertility in vitro and in vivo (AI trial) with both diluted and frozen-thawed semen. We will also try to analyze the problems and limitations related to the interpretation of boar sperm tests.

Keywords: Porcine fertility; Sperm fertilizing ability; Boar sperm; Sperm motility; Acrosome morphology

1. Introduction

The prediction of sperm fertilizing ability has great economic importance for breeding herds when artificial insemination is used, since it leads to the selection of only those boars

* Tel.: +34 968 364655; fax: +34 968 364147.
E-mail address: jgadea@um.es.

0093-691X/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2004.09.023

with a good reproductive performance. At the moment, however, many unsolved problems remain. Fewer tests relating to sperm factors and fertility have been developed for boar sperm than for other domestic species, such as bulls. In most of such tests, the relationship has been found to be of relatively little significance [1–5], due in part to the many factors involved in the fertilization process [6] and in the fertility of sows.

Fertilization is a complex process with many different sequential events. Sperm tests usually assess only one or a few of these events. In this review, we will try to analyze the three most important aspects in the study of the biological relationship between sperm factors and fertility. These are the assays used, the factors related to the female and the insemination process, and the methodology used to study the relationship.

2. Sperm assays

One of the main goals in spermatology is the development of new assay methods to detect the characteristic alterations in sperm that are evidence of reduced fertility [7–11]. A great variety of sperm tests have been used. The classical methods are based on the examination of the structure of the cell and are poor in predicting subsequent sperm fertility because only the samples with markedly poor quality can be detected. To solve this problem, new procedures have been developed for the evaluation of sperm functionality, including the study of oocyte–sperm interaction.

2.1. The classical spermiogram

The classical method for semen evaluation is based on the application of a battery of tests that are simple to perform and at relatively low cost. In general, they measure the volume of ejaculate, the sperm cell concentration, the total number of sperm in the ejaculate, the progressive motility, the percentage of viable cells, and the morphology. The classical laboratory methods are usually insufficient for predicting fertility (farrowing rate and litter size) [1,12,13], but the use of combined tests can provide additional information for evaluating sperm quality [1,2].

Sperm production as reflected in sperm concentration, semen volume, and number of sperm in the ejaculate is an important parameter in pathological studies when altered testicular function is clearly related to reduced fertility. However, in case of more subtle differences in fertility, the relationship between fertility and litter size and the volume, concentration, and number of sperm in the sperm-rich fraction is very poor [12,13]. A large number of factors affect sperm production (breed, age, housing, etc.) [14], but there is no clear relationship between these seminal parameters and the fertility of boars used in AI [15–17].

An evaluation of motility is the most widely used test because it is simple, quick, and inexpensive. It is a good indicator of the intactness of the membranes and functionality. Despite severe limitations, motility seems an efficient seminal parameter because it is significantly related to the farrowing rate and total number of piglets born, so that it is included in all the multivariate models as a significant component [12,13,18]. In general, however, the correlation coefficient and the percentage of the variations in fertility that are

explained are low. The results in the literature regarding the relation between motility and fertility are contradictory [17–24]. These contradictory results may be caused by large experimental differences. Nevertheless, sperm motility is a subjective measure that depends on the individual observer. The precision with which estimates of sperm motility are made is obviously important. The correlation coefficient is higher when an average of multiple subjective estimates is used, rather than simple estimates [6], thus reducing the sampling errors. A computer-assisted sperm analyzer (CASA) has been used to solve this problem and a significant correlation with fertility was found [23,25]. However, the CASA system is not exempt from other technical and human errors [26,27].

Sperm morphology is another interesting parameter and appears to be related to fertility, at least as a tendency. Many morphological anomalies have been related to cases of infertility [28]. In standard semen analysis, this provides information on the status of spermatogenesis, and it can facilitate the selection of boars for AI programs [29]; it also allows workers to better assess the intensity of stress produced by a high frequency of semen collection [30]. An inverse relationship has been reported between the number of morphological anomalies and fertility [12,15,17,20,29,31]. In this connection, Xu et al. [17] showed how morphology explained a large part of the variation in litter size in a commercial setting ($R^2 = 0.59$). Recently, Hirai et al. [25] have used automated sperm morphology analysis (ASMA) and described a significant relationship between sperm head dimensions and fertility.

Intactness of the spermatozoa plasma membrane is a prerequisite for suitable sperm metabolism and function [32]. The different methods for evaluating the plasma membrane include eosin–nigrosin staining and various fluorescent stains (propidium iodide, carboxyfluorescein diacetate, SYBR-14, Hoechst 33258, etc.). However, this information on membrane structure is not closely related to fertility [12,13,24], perhaps because it provides information about the viability of the sperm but not about its functionality (capacitation process, acrosome reaction, sperm binding, etc.).

Several authors have described how high numbers of altered acrosomes are related to problems in fertilization. However, the correlation coefficients between NAR and fertility were not high [16,18–20,33,34]. In theory, a test of the functionality of the acrosome reaction should be more accurate in predicting potential fertility.

With the classical spermiogram it was possible to detect very low quality samples and eliminate them as being associated with poor fertility. However, this is not an accurate way to distinguish samples with excellent fertility from those with medium fertility [12]. This could be related to the very limited variation in these parameters in mature fertile boars [17], or to the fact that these tests do not properly evaluate the functionality of the sperm. According to Flowers and Turner [35], the common microscopic estimates of semen quality are good qualitative but poor quantitative indicators of semen fertility.

2.2. *New sperm assays*

Once it was recognized that the standard spermiogram does not provide reliable diagnostic information about boar fertility, it was necessary to improve the analytic procedures. The new sperm assays try to explore the functional capacity of the spermatozoa. Muller [9] defined a sperm function test as a laboratory analysis of the

cellular processes exhibited by spermatozoa between the time they leave the seminal fluid and the final step of fertilization. These tests attempt to determine the ability of sperm to capacitate and fertilize.

In that sense, many tests have been developed to evaluate these functional attributes. Diverse fluorescent dyes have been used to bind to different regions of the sperm in order to evaluate special functional characteristics of the cell [36], often assisted by modern flow cytometry that allows rapid counting of large numbers of cells [37,38]. Among these functional tests, the study of the sperm membrane or plasmalemma is of particular importance since a biochemically active membrane is required in the process of capacitation, the acrosome reaction and the binding of the spermatozoon to the oocyte surface [39]. These tests of membrane function, like the hypoosmotic swelling test (HOS), can determine whether an intact membrane is biochemically active. Drevius and Erikson [40] first described the osmotic swelling of bull spermatozoa in response to a hypoosmotic medium because of the influx of fluid into the spermatozoa. Jeyendran et al. [41] later adapted and applied the HOS to human spermatozoa. Vázquez et al. [42] adjusted the protocol to boar spermatozoa with different hypoosmotic solutions, osmotic pressures, and times. More recently, the HOS has been successfully related to in vivo [12,33] and in vitro fertility [43]. The HOS has also been used to evaluate the sperm functionality during in vitro storage [44] and after capacitation procedures [45]. It has been widely used in both human andrology and domestic species. Up to now, however, its use for boar spermatozoa has been limited.

The HOS is a simple, easy to perform, inexpensive, and repeatable assay. The HOS shows good reproducibility (intra-assay CV 4%) and no differences among technicians performing the test [33]. The test can be run with different solutions, including a commercial extender, in a range from 50 to 150 mOsm and 5 to 30 min at 37 °C [42]. Counting must be done under a phase contrast microscope to improve the accuracy. Nevertheless, some problems appear in the evaluation of samples with a high number of morphological anomalies, such as folded and coiled tails [46] and when sperm with an intact plasma membrane are unable to react to moderate osmotic pressure [47].

In relation to other sperm parameters, the HOS score is usually lower than the motility and plasma membrane integrity [12,33,42,44], and good correlation coefficients have been observed with this parameter [33,44,46]. The relationship to fertility and litter size is significant [12,33]. A positive relationship has also been found with fertility in vitro [43]. However, in our studies, the HOS did not provide significantly more information for the prediction of fertility than other classical methods [12,43]. Another sperm test that involves the use of a hypoosmotic medium is the osmotic resistance test (ORT) described by Shilling and Vengust [48]. This test evaluates the percentage of intact acrosomes after incubation in an iso-osmotic medium (300 mOsm/kg, 15 min, 39 °C) and a hypoosmotic medium (150 mOsm/kg, 120 min, 39 °C). This yields predictions of fertility that are similar to those with other membrane tests [12,43].

Other functional tests explore the cellular metabolic activity [49]. The ATP concentration has been suggested as a reasonable test of sperm health [50], but no significant correlation has been found with fertility [12,24]. The mitochondrial activity can be analyzed by some specific fluorescent stains like Rhodamine 123 or JC-1 [44], but this activity has not been related to on-farm fertility.

With regard to capacitation and the acrosome reaction, Tardif et al. [24] related the capacitation status as measured with the chlorotetracycline stain to fertility, but obtained a significant relation with the percentage of sperm having an AR (acrosome-reacted) pattern of membrane staining only when low numbers of sperm per dose were used in the insemination trial. Holt et al. [26] also showed that the incidences of spontaneous, ionophore- and zona-induced acrosome reactions were significantly higher in the ejaculates with low litter size.

The structure and functionality of the sperm nucleus are of great importance for the subsequent fertility. The integrity of the chromatin has been measured by flow cytometry in the sperm chromatin structure assay (SCSA) [51]. In the boar, the integrity of the chromatin is directly related to the fertility [52,53]. The integrity and condensation of the chromatin is affected by the freezing procedure [54,55]. However, it was not possible to show a direct relationship with in vitro fertility [55]. Warberski [56] will discuss the application of these assays during this conference.

Finally, some other molecules are implicated in the fertilization process, such as seminal plasma proteins, heat shock proteins, etc. In the near future, assays of these molecules may lead to the identification of differences that could be associated with fertility (reviewed by Braundmeier and Miller [10]).

2.3. IVF systems

Among all the sperm tests that have been developed, IVF tests are the most suitable for assessing overall sperm function during fertilization. The binding and penetration of the zona pellucida is one of the most important barriers the spermatozoa must overcome in the fertilization process [32]. Also, the interaction with the oocyte plasma membrane appears to explain much of the variability in sperm fertilizing potential among fertile boars [57]. Hence, assays that include a study of gamete interaction may lead to a better way to predict male fertility than the routine laboratory evaluation of semen [58–60].

In the pig, a number of assays have been shown to be good tools for evaluating the fertilizing capacity of boar semen: the sperm penetration assay (SPA) with zona-free hamster oocytes [16,21,57], sperm-zona binding to intact pig oocytes [61] or to stored pig oocytes [62], the hemizona binding assay [63], and homologous in vitro fertilization using in vitro matured oocytes [17,18,34,35,64] or using porcine zona-intact oocytes at the germinal vesicle stage [65,66]. The last-named homologous in vitro penetration assay (hIVP) has been shown to be a good tool for evaluating the fertilizing capacity of fresh boar semen [12,67] and for assessing stored boar semen [68]. The possibility of using zona-intact pig oocytes at the germinal vesicle (GV) stage (immature oocytes) in a hIVP assay of boar sperm fertility would facilitate the collection of female gametes, thus shortening the time required for the assay by saving time spent in completing in vitro maturation [65,66]. On the other hand, the hIVP only properly evaluates the penetration process, the first step in fertilization; this is followed by other events that could be evaluated with matured oocytes, like pronuclear formation and early embryonic development [59].

When IVF systems are employed, the penetration rate is the commonly used measure of the boar sperm fertilizing ability. The average number of sperm per penetrated oocyte does not reflect the normal events during fertilization in vivo, but may provide a useful estimate

of the number of spermatozoa with high fertilizing ability. A strong correlation has been found between the number of sperm per oocyte and the penetration rate ($r = 0.55$–0.71, $p < 0.001$) [12,43]. Some other parameters are also measured, such as the number of attached spermatozoa, the percentage of male pronuclear formation, the number of embryos developed, etc. The best parameter in IVF assays would be the capacity for viable embryo production in vitro [59]. These studies have been developed in the bull with a significant relation between cleavage and blastocyst formation rates and fertility (non-return rates) [69]. In the pig, the difficulties in embryo production in vitro have so far prevented this relation from being evaluated [70,71].

In our experience with IVF systems, the penetration rate has been retained in the best logistic regression models relating to fertility and was significantly correlated with litter size in fresh [12] and frozen semen [18]. On the other hand, Xu et al. [17] obtained a good correlation between the number of sperm attached per oocyte and litter size. In humans, a large number of studies have been carried out to detect a cut-off value in the SPA (zona-free hamster ova sperm penetration) in vitro penetration test for the prediction of fertility [72,73]. In the pig, Gadea and Matas [43] have determined the cut-off value in the prediction of fertility for the hIVP test. In this study, the best cut-off value for the in vitro penetration rate to forecast in vivo fertility was found to be 75%; this yielded 74.16% true positives and 25.84% false positives in the prediction of the in vivo fertilizing ability.

The literature regarding the influence of sperm factors on the success of in vitro fertilization is confusing [21,61,65,74]. The contradictory results may be caused by the large experimental differences, by the low number of ejaculates or IVF trials analyzed, by a high number of sperm per oocyte in the IVF system, which could mask the relation, or by the preselection of the ejaculates.

Generally, little relation has been found between the sperm parameters and the in vitro penetration ability, and when the relation was significant, the regression coefficient was low [16]. Few single sperm parameters appear to correlate significantly with in vitro penetration, especially when the semen samples are within acceptable ranges of normality. It has also been suggested that the lack of correlation between conventional semen quality tests and sperm penetration assays is due to the fact that these assays measure different aspects of sperm viability and fertilizing capacity. However, in our experience, we have used a large number of ejaculates and have not preselected them, so that a large number of seminal parameters have been found to be related to the in vitro penetration ability; when the ejaculates were preselected, the number of significantly related parameters was lower [43]. Most of the values studied are significantly related to the in vitro penetration rate, except for the sperm concentration and eosin–nigrosin staining. The ATP concentration in spermatozoa correlates with the penetration rate and the number of sperm per penetrated oocyte; however, ATP measurement appears to have little diagnostic value in predicting the fertilizing capacity as evaluated by multivariate stepwise analysis, as previously described in humans by Chang et al. [75]. With regard to sperm quality, progressive sperm motility seems to be a good indicator of sperm fertility and is highly correlated with oocyte penetration rates [35,64] and with the number of sperm per penetrated oocyte [16]. In our own paper [43], these motility parameters also appear in the multivariate models. However, in other studies, no correlation has been found between the penetration rate and sperm motility [65,76].

The acrosome reaction is required for sperm penetration through the zona pellucida of the oocyte and subsequent fusion with the plasma membrane. However, no relationship has been found between the penetration rates of ovulated oocytes and the maximal percentages of reacted acrosomes (PNA lectins and triple stain technique [74]). Similarly, Lynham and Harrison [62] found no relation between the strength of sperm-zona binding and acrosomal status as assayed by fluorescein-conjugated peanut agglutinin. In these cases, the use of optimum assay conditions for sperm penetration may have resulted in a loss of sensitivity in detecting a specific correlation between sperm penetration and acrosome status. The NAR results in our studies were highly correlated with penetration rate and appear in the stepwise multiple regression models [43]. The stepwise multiple regression models obtained explain a high percentage of the total variation with four (linear) or seven (logistic) variables. These results suggest that in vitro penetration failure or success cannot be explained by the alteration of any simple sperm characteristic, although a consideration of several characteristics in association may permit the prediction of failure or success in penetration. In summary, these results have shown that almost every studied parameter of boar semen is significantly different between penetration successes and failures, but that most of them are interrelated. These findings emphasize the complexity of sperm functions and the difficulties in assessing boar fertilizing ability.

Equally, some of the assays that study the spermatozoon–oocyte interaction have been shown to be good tools for evaluating the fertilizing capacity of frozen boar semen [16,18,77] and indicate that it would be very useful to evaluate the freezing procedures [18,55,78,79]. In our studies with frozen spermatozoa [18], the conventional seminal parameters were insufficient to discriminate between good and bad boars in relation to fertility. On the contrary, the penetration parameters (penetration rate and number of sperm per oocyte), measured in an IVF system, were more precise in the prediction of subsequent fertility. On the other hand, it turned out to be more difficult to predict the litter size [4], probably due to important maternal and environmental effects (ovulation rate, fertilization rate, relation between insemination and ovulation, etc.). Thus, only a small number of the classical sperm parameters are related. However, with a multivariate model, only two parameters, the penetration rate and the number of sperm per oocyte, are needed to explain nearly 80% of the variability [18].

Moreover, IVF seems to be a good tool for evaluating the quality of frozen-thawed boar semen prior to its commercial use, for verifying the storage quality of banked semen, and for the assay of new sperm freezing procedures, since it is the most precise method for estimating the potential fertilizing ability.

In summary, IVF systems can be used successfully for evaluating the fertilizing capacity and are more accurate than other methods. At present, however, these analyses are expensive and time-consuming and are, therefore, far from satisfying on-farm commercial requirements.

3. Factors associated with the female and the insemination process

Fertilizing ability is commonly measured as the percentage of sows or gilts conceiving or farrowing following artificial insemination [6]; with some limitations, these measures

are indicative of the efficiency with which eggs are fertilized with spermatozoa that are capable of sustaining embryonic development [80]. The most valid assessment of boar fertility is to obtain viable pregnancies and normal offspring following in vivo insemination. However, field trials of semen fertility are also imprecise because of the high variability associated with the female [81] and with the conditions of insemination [6]. Technical expertise in estrus detection and competence to carry out the insemination can have a major impact on the success rates [23]. These are the types of factors that contribute to the group of ''other sources of variation'' [11]. The sow effect is higher the more homogeneous and higher the sperm quality. Also, it is a major inherent variation in estimating fertility because of the binomial variation in the dichotomous parameters of pregnancy or farrowing. Consequently, it is necessary to inseminate a large number of females to obtain significant levels. This implies spending a great deal of time, high cost and a considerable delay before the fertility information is obtained. During this time, there could be significant changes in the boar fertility.

With the aim of reducing the time required for the study, alternatives are employed, such as the use of non-return rates, the recovery and evaluation of ova/embryos after a fixed number of days post-insemination [82,83], or indeed the recovery of fetuses after some weeks of gestation [24]. Fertilization rates, the proportion of normal embryos and the number of accessory sperm have been studied. In embryos recovered on days 3–6 post-insemination, the accessory sperm are thought to represent the population of fertilization-competent spermatozoa (reviewed by Saacke et al. [84]). This implies that accessory sperm fulfil the structural and physiological requirements necessary for traversing the female tract as well as for ovum recognition, binding and partial penetration of the zona pellucida. In this sense, it is concluded that the number of accessory sperm is a more sensitive parameter for evaluating the fertilization capacity than the fertilization rate of the oocytes [83,85].

The use of IVF leads us to analyze a great variety of factors associated with the fertilization process [71] and eliminates some of the factors that affect in vivo female fertility. However, IVF does not allow or require the expression of the complete repertoire of attributes that must be functional in sperm deposited in a female [11]. Consequently, we must be conscious of the limitations of the in vitro system and cautious in extrapolating from in vitro observations to in vivo results [86].

To minimize the effect of female variability, the heterospermic test was developed in the 1950s by Beatty in rabbits [87] and in cattle [88]. In the pig, this test has been used successfully for both fresh [19,21,57,85,89] and frozen semen [16]. The test is based on the insemination with the same number of sperm from two boars. In this way, we maintain the sperm in a competitive situation in which the most capable spermatozoa will penetrate the oocyte and a higher proportion of piglets will be produced. Results from the use of the heterospermic procedure indicate that the variation in fertility among boars is large [57] and eliminate the confounding factors, such as female fertility, season, nutrition or management practices [85].

This test leads to a reduction in the number of sows in the trials, but has some limitations. First of all, the resultant fertility is a relative index depending on the boar used in the trial; the test can obviously only be carried out in a limited number of boars and it is difficult to translate the index to parameters of fertility or litter size [4]. On the other hand, for the identification of paternity, it is necessary to determine, in a reliable way, a

phenotypic character (coat color, blood groups, isozymes, etc.) or polymorphic DNA markers [85]. At the moment, therefore, this technique is limited to experimental trials.

One of the main factors that could be the cause of the differences in the experimental results is the number of spermatozoa used in the insemination dose. A large number of sperm per dose could compensate any boar infertility factor and thus mask the relation to sperm quality [3,4,18,24]. Hence, experiments using a reduced number of sperm per dose would give a better discrimination of the variations in semen quality from different ejaculates and might clarify the relationships between in vitro measurements and in vivo fertility [18]. However, this reduction in the number of sperm would deviate very far from the commercial conditions. Another important factor affecting fertility is the synchronicity between insemination and ovulation. Because of the high variability in the interval from the onset of estrus to the initiation of ovulation (reviewed by Flowers and Esbenshade [90]), some alternatives have been employed to reduce this variability, like the induction of ovulation by administration of hormones in gilts [24] or the control of sow ovulation with the use of ultrasonography [91,92].

4. Methods for relating sperm quality and fertility

Information from semen analysis is used to predict the likelihood that a group of gilts/sows will conceive after AI. In this sense, it is only possible to provide an estimate of probability; there can be no certainty. The probability is influenced by a host of factors, including semen quality. The problem is made worse by variations in sperm parameters among different ejaculates from the same boar.

Considering that many assays test only a single attribute, it is unlikely that the fertility will be predicted accurately because many successive steps must occur for fertilization to succeed. The use of multivariate analysis would help to discriminate potential fertility because this combines the functional information regarding different capacities of the spermatozoa. A combination of selected semen tests, therefore, yields a higher accuracy than a single test in the prediction of fertilizing capacity [1,2].

Another conflicting situation is due to the fact that laboratory assays examine a representative sample from all of the spermatozoa in the ejaculate. However, only a selective subpopulation of the spermatozoa in the boar ejaculate fertilizes the oocytes in the gilt/sow. It is, therefore, necessary to develop methods for analyzing this subpopulation [32].

Different statistical models have been used to relate fertility and seminal parameters. Logistic regression is a robust method for the analysis of categorical data (fertility rates); it is better than linear regression of transformed data, but the former is difficult to manage with the odds ratio. In practice, both models are equally effective in relating fertility and seminal parameters. As expected, there is a high degree of correspondence between the different methods, and it has been shown that ‘‘biological evidence is related with facts, not with any peculiar statistical test’’ (Romar A, personal communication). Besides, the determination of cut-off values for seminal parameters has been described in the literature more as the result of empirical work than in relation to a scientific approach to the problem.

A statistical discriminant analysis of the data could give some valuable information. However, because of the poor relation between seminal parameters and fertility, the sensitivity and specificity of such a cut-off is very limited.

Some possible causes of the lack of a relation between fertility and seminal characteristics could be related to the fact that certain sperm characteristics cannot be analyzed by the classic spermiogram. In this connection, Saacke et al. [93] define the concept of compensatable sperm traits as those that do not affect fertility if a large number of spermatozoa are present in the insemination dose. The non-compensatable sperm traits are the sperm characteristics or deficiencies that are associated with sperm that are incompetent for fertilization (those sperm that can initiate but not complete the fertilization process or sustain early embryogenesis). These non-compensatable sperm traits are presumably related to alterations in the DNA and nucleoproteins in sperm chromatin [52]. The compensatable sperm traits make the sperm unavailable for fertilization (reviewed by Evenson [53]) and can be assessed by the classical spermiogram.

Another factor that is implicated in this experimental difference is the preselection of the ejaculates. Under commercial conditions, the ejaculates are usually selected on the basis of motility, so only those with motility above a reference value are used. This situation drastically limits the variability of the sperm parameters. In a previous study, when no preselection was done, higher correlations were found [12], but the preselection that is done under commercial conditions could reduce this relation, as has been demonstrated for in vitro fertility [43].

5. Conclusion

The study of the relationship between fertility and boar sperm factors is a difficult adventure because of the complexity of the process. Nevertheless, important steps have been taken in the last decades. New functionality tests and IVF systems have been used successfully for evaluating the potential fertilizing capacity. At the moment, however, these analyses are expensive and time-consuming, and are, therefore, far from satisfying on-farm commercial requirements. Sperm analysis under commercial conditions leads to the detection of ejaculates with very poor quality (associated with poor fertility). The selection of the samples, the high quality of the semen and the large number of sperm used in the AI programs mask or reduce the variability. The detection of fertility differences associated with seminal factors is, therefore, very unlikely.

In the near future, the generation of new knowledge in molecular and genomic technology will help us to evaluate the potential fertility of boar ejaculates more accurately.

Acknowledgements

The author's work has been made possible by grants from the Spanish Ministry of Education and Culture, the Spanish Ministry of Science and Technology, and the Seneca Foundation, Región de Murcia, Spain.

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