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ScienceDirect
Theriogenology 70 (2008) 1260–1268
Theriogenology
www.theriojournal.com

Review

# Boar semen variability and its effects on IVF efficiency

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**Abstract**

In vitro fertilization (IVF) in pigs is still considered sub-optimal, due to the occurrence of polyspermy, as well as the inter- and intra-boar variability in sperm characteristics. Numerous studies have investigated the relationship between fresh and frozen-thawed semen parameters, such as motility, morphology and viability with in vitro fertility in order to develop methods of selecting boars for use in IVF. These studies have clearly shown that sperm parameters have limited value in predicting IVF efficiency. On the other hand, it has been demonstrated that the requirements of boar sperm during co-incubation with the oocytes (sperm:oocyte ratio, substances added to the fertilization medium and co-incubation time) vary among boars. Preliminary assays required for individual males will be discussed with the objective of reaching maximum efficiency of in vitro fertilization.


*Keywords:* Boar; In vitro fertilization; Polyspermy; Frozen semen; Efficiency

**Contents**



## 1. Introduction

For many years, there has been considerable interest in improving porcine reproductive technologies for both biotechnological and biomedical applications. Functional in vitro maturation (IVM), in vitro fertilization

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0093-691X/$ – see front matter 
doi:10.1016/j.theriogenology.2008.06.004

(IVF) and culture (IVC) systems could produce large numbers of transferable in vitro produced (IVP) embryos. These are pre-requisites for further development of research areas such as transgenesis, xenotransplantation and the production of offspring of a predicted sex. This interest has generated IVM–IVF–IVC procedures that alleviated the initial problems of poor male pronuclear formation and the four-cell block, and have successfully produced embryos with acceptable pregnancy rates and litter sizes after their transfer to recipient animals [1–8]. However, despite this extensive research, we are still faced with the unresolved problem of a high incidence of polyspermy resulting from IVF. In fact, the efficiency of IVF (percentage of monospermic oocytes from total inseminated) still remains at 40–50% in most of laboratories [6,9–14]. Furthermore, since polyspermic porcine embryos from IVF cleave and develop to the blastocyst stage at the same percentage as normal embryos [15,16], little information is obtained from monitoring blastocyst formation. Accordingly, it is necessary to visualize pronuclei formation by staining, or cytogenetic examination by chromosomal karyotyping, to actually assess the incidence of normal fertilization.

Causes of polyspermic fertilization include the quality of the matured oocytes [17–20], semen quality at fertilization [21,22] and the IVF conditions (revised by Abeydeera [23]). Nevertheless, variation among boars, ejaculates within boars, as well as among different fractions within the same ejaculate, appears to influence the incidence of penetration and polyspermy [24,25]. The use of frozen-thawed sperm eliminates variation between trials, but still does not lead to acceptable rates of monospermy, and a single IVF protocol may not be optimal for different batches of frozen-thawed sperm. This implies that before using a batch of frozen-thawed semen for IVF it is imperative to empirically define the optimal IVF conditions to obtain maximum efficiency.

This review focuses on how boar semen variability and the co-incubation conditions affect IVF efficiency and what preliminary assays are required for each batch of semen.

## 2. Breeds, ejaculates and fractions of the ejaculate

Large variation among boars has been reported in fertilization rates with fresh [22,24,26,27] and frozen-thawed sperm [28,29]. Boar and ejaculate variation has been a problem in maintaining acceptable efficiency in vitro. The understanding and/or removal of these sources of inter- and intra-boar variability, would lead to more standardized IVF procedures.

As mentioned above, not all sires respond equally to in vitro fertilization. Suzuki et al. [30] reported, using fresh and frozen-thawed semen from 15 boars of 3 different breeds (5 boars/breed), that penetration and polyspermy rates were more variable among the breeds than among boars within breed. Spermatozoa from Large White boars penetrated at significantly higher rates than those from Landrace and Duroc breeds, but the resulting fertilization was also more polyspermic. Furthermore, irrespective of breed, important differences among fractions within the same ejaculate have been demonstrated by Xu et al. in oocyte penetration and polyspermy [24,25]. These researchers defined three ejaculate fractions: the sperm-rich portion, that contained the greatest proportion of viable spermatozoa (F1); the least concentrated portion after the first sperm-rich fraction (F2); and the most concentrated portion of the second sperm-rich fraction (F3). When the effect of these three fractions on IVF was investigated, they found that spermatozoa from the seminal plasma-rich fraction (F2) had significantly lower fertilizing ability than those from the sperm-rich fractions. Although there were no differences between the two sperm-rich fractions (F1 and F3), fertilization tended to be lower in the second (F3) sperm-rich fraction, so they suggested the use of F1 only, to standardize IVF conditions and to reduce variability among different ejaculates collected from the same boar.

The three fractions of the ejaculate used by Xu et al. [24,25] showed approximately 85% of progressive motility and less than 8% morphologically abnormal spermatozoa. Therefore, in an attempt to identify whether the variability in functional capacity of the three ejaculate fractions originated from the seminal plasma or the spermatozoa themselves, the same research group incubated sperm pellets from the first sperm-rich fraction (F1) with each of the three fractions of seminal plasma from the same ejaculate [31]. Differences encountered in penetration rate between sperm co-incubated with F1 seminal plasma (∼75%) compared with F2 (∼49%), suggested that different seminal plasma fractions contain substances with varying effects on spermatozoa and on their ability to fertilize the oocytes in vitro. In general terms, boar seminal plasma contains high concentrations of estrone, estradiol-17β, estrone sulphate and specific proteins involved in sperm capacitation and gamete recognition as well as binding [32–35]. From Zhu et al. [31], it seems that F1 seminal plasma may contain particular