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Development of a sperm binding assay for boar semen

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Development of a Sperm Binding Assay for Boar Semen

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ABSTRACT (100-200 WORDS):

Sperm-egg binding assays have been developed as diagnostic tests to determine fertility in humans, horses, and cattle. The aim of this study was to develop a similar assay for use in porcine species. Porcine oocytes were obtained from slaughterhouse ovaries. The oocytes were dried on to microscope slides, where they could be stored for up to three months. Whole semen was obtained the day after ejaculation, diluted, and co-incubated with the oocytes on the slide for 10 minutes at 37C. The sperm-egg complexes were rinsed and stained with an ultraviolet DNA stain. The number of sperm bound per oocyte was counted and recorded. As conclusive farrow rate data becomes available, the binding data will be analyzed to determine if there is a statistically significant correlation between binding and overall fertility in the porcine species.

Introduction

Artificial Insemination (AI) as used in commercial livestock operations allows widespread use of genetically superior males. In modern swine operations, a boar used with AI could potentially be mated to fifteen to twenty sows per semen collection. Hence, the ability to predict the fertility in selected males would enhance production efficiency by removing those males with reduced fertility.

In humans, horses, and cattle(Fazeli *et al.* 1993) a positive correlation has been shown between the number of sperm bound on the outer egg coat (zona pellucida) and fertility. Fertility in swine is usually indicated by the number of bred sows which farrow a litter. This is referred to as the farrow rate (FR). Little information exists about whether or not a correlation exists between sperm-egg interaction and fertility in swine.

The objective of this project was to determine if the sperm-egg assays of other species could be adapted for the pig. It is known that pig gametes are manipulative for in-vitro work , and it was assumed that certain aspects of the published assays of other species would require slight modification for assay development.

Materials and Methods

Oocyte Preparation

Ovaries were obtained from slaughterhouse gilts and follicular fluid was pooled by passing the ovaries over a ganged razor blade apparatus. The follicular fluid was filtered through a 500 um mesh, a 200 um mesh, and collected on a 50 um mesh(Gwatkin, *et al.* 1980). The oocytes were rinsed with phosphate buffered saline (PBS) and placed in PB1 media. Cumulus cells were removed by passing through a narrow bore pasteur pipet. Intact oocytes free of cumulus cells were collected and stored at 4° C in buffered ammonium sulfate until ready for slide preparation. Oocytes were stored for up to four weeks.

Semen Sources and Analysis

Whole semen diluted 1:10 with TALPS was obtained from boars one day after ejaculation. Motility was determined via light microscopy within 24 hours of semen arrival. Semen samples with <50% motility were not used in the binding assay. Semen was stored at 21° C. Immediately before the assay, the semen was diluted 1:5 in Whittens media.

Slide Preparation

The slides were etched with a line horizontally down the middle. The oocytes were rinsed in distilled water and placed on the slide with a narrow bore pasteur pipet. Sixteen oocytes were placed on each slide, immediately above and below the etched line. The

oocytes were allowed to dry on the slide overnight on a 38° C warming plate. The slides were stored at room temperature in a dry place for up to three months.

Zona Pellucida Binding Assay

2 ul of dilute semen was placed on top of each oocyte on the slide. The slides incubated for 10 minutes at 38.8° C and 5% CO_2. Slides were removed and dipped in distilled water fifteen times to remove sperm that were loosely attached but not bound. Slides were placed in 1mg/ml Hoechst 33342 stain for one minute. Slides were viewed under a UV microscope and the number of sperm bound per oocyte were counted and recorded.

Data

	H 8190	H 10626	H 11270	S1 30776	S2 40368	Z 10086	Z 11488	Z 11489
Number of Replicates	3	3	3	3	3	3	3	3
Number of Observations	45	41	61	47	42	46	42	46
Percent Motility	83	72	88	78	85	82	83	73
Average Binding	16.7	41.6	18.5	24.4	43.3	26.5	17.8	43.6

Results and Discussion

Microscopic examination of sperm for such parameters as motility, concentration, and abnormalities has been used to determine sperm quality. However, these criteria offer limited value in predicting the fertility of the male in question. The only direct correlation noted between motility and fertility is found in cases of extremely low motility (this is due to the inability of the sperm to reach the oviduct, the site of fertilization). In swine practices, AI is becoming more and more important. It is paramount, therefore, to be able to predict the fertility of the boar as he may be mated to fifteen to twenty sows per semen collection. In humans, horses, and cattle, sperm-zona pellucida binding assays have been developed to predict fertility.

We found that oocytes recovered from the slaughterhouse and dried on to microscope slides was a useful and novel way of effecting sperm-egg binding. Sperm and egg were co-incubated for ten minutes at 37° C, rinsed, and stained with a DNA stain. The slides with fixed sperm-egg complexes could be viewed at a later more convenient time. Up to eighteen eggs could be placed on one slide allowing the user more efficiency.

Noted differences were observed in boars with respect to sperm-egg binding rates. Preliminary farrow rate data suggests that boars with differing sperm-egg binding have differing farrow rates. As more conclusive farrow rate data becomes available, the results will be analyzed statistically to note if significant differences exist and to predict the value of the assay as a diagnostic tool. It is important to analyze the semen profiles of those boars which have been mated to a relatively large number of sows (>50) to be able to draw statistically significant conclusions. It is probable that different genetic lines of pigs will have differences in sperm-egg binding capacities. Therefore, each genetic line may have to be characterized to determine a "sperm-egg binding average" that is relative to that line. As more boars are examined across lines, that information will become available.

References

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