The Role of the Acrosome in Prediction of Fertility

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Story in Brief

It has been the desire of reproductive physiologists, veterinarians and bull studs to find a reasonably reliable indicator of the fertility of bulls. This has been a disappointing search for several years. Some indicators of ejaculate quality have been used for years to grossly separate and identify bulls or ejaculates of "acceptable" quality. No single indicator is highly indicative of fertility.

Research in recent years has developed means to ascertain the morphology of the acrosome which is a cap-like structure over the anterior half of the sperm nucleus. Our research measured several ejaculate characteristics, including the acrosome state, and utilized these in attempting to build a fertility prediction equation. The observations summarized in the report indicate that several pre-freeze and post-freeze ejaculate characteristics can be combined to give a reasonable prediction of fertility.

Introduction

The reports of many researchers over several years have shown several ejaculate characteristics to be associated to some degree with fertility of the male. (Lasley and Bogart, 1943; Shaffer and Almquist, 1949; Erb et al, 1951; Bishop, et al, 1954; Munroe, 1961). However, progress in developing a system for predicting fertility has been slow because no single measurement or series of measurements has sufficed in accurately evaluating fertility (Saacke, 1970).

It is known that the acrosome is important in the fertilization process, however, the exact mechanisms which are regulated or moderated by acrosome function are not well defined. Research on the acrosome has been renewed in recent years by the development of means that allow the morphology of the acrosome to be examined (Saacke and Marshall, 1968; Wells and Awa, 1970). Recent reports (Saacke, 1970; Saacke, et al, 1968; Saacke and White, 1972) indicate that the state of the acrosome in a population of cells may be a useful indicator of the potential fertility of that population.

The objective of this study was to evaluate a number of sperm characteristics and determine their significance in prediction of fertility, and develop a fertility index utilizing these characteristics.

Procedure

Forty-two Holstein bulls in the progeny test unit of American Breeders Service, De Forest, Wisconsin, were utilized in this study. All bulls were approximately one year of age at the time of initial ejaculate collection, were housed and handled similarly, and were fed a routine growth ration throughout the conduct of the study.

Two ejaculates were collected and processed for freezing once weekly from the bulls until 520 ½cc ampules, containing 7 to 13 million live sperm, were obtained. Five to eight collections were typically required to get the desired number of ampules in storage. This frozen semen was randomly distributed to cooperating herds across the United States, however, no bull was to be used more than four times in any given herd. It was projected that data on 150 to 200 services to each bull would result from the described distribution.

Several measurements were made on sperm characteristics of each ejaculate, both pre-freeze and post-freeze. Percent live cells was estimated microscopically and by slides differentially stained by the method of Hancock (1952). Two hundred sperm cells were examined per slide to determine the percent live. Acrosome characteristics were determined from 200-cell-counts on differentially stained smears (Wells and Awa, 1970). Cells were first classified as normal or abnormal morphology and then as aged or non-aged acrosomes within these classifications. From the slides prepared, the following pre-freeze and post-freeze percentages were determined for each ejaculate:

1. Live cells
2. Aged acrosomes
3. Normal morphology
4. Normal cells with aged acrosomes
5. Normal cells with nonaged acrosomes
6. Abnormal cells with nonaged acrosomes
7. Abnormal cells with aged acrosomes.

A microscopic estimate of percent motile cells was made immediately post-freeze and one month post-freeze. Ampules were thawed in 5° C. water for 5-7 minutes and a small drop of semen was placed on a slide, warmed to 37° C. and the percent motile cells was estimated to the nearest whole percent.

These 16 variables were analyzed to determine (1) their contribution to the fertility of the animal, and (2) the relative weight that should be given to the significant variable in computing an estimate of fertility.

Results and Discussion

Approximately 520 ampules of semen per bull were distributed across 60,000 cooperating herds by ABS. There was a total of 5,976 first services with an average of 142 services for each of the 42 bulls. The number of services ranged from a low of 110 to a high of 182 per bull. Fertility on a 60-90 day non-return to first service basis for the 42 bulls ranged from 53.3 to 82.6 percent with an average across all bulls of 70.7 percent.

Table 1 presents a summary of the average pre-freeze and post-freeze evaluations of sperm characteristics measured in this study. It will be observed, first of all, that the average percent live sperm decreased from 81.5 percent pre-freeze to 32.5 percent post-freeze. This is in good agreement with the industry observation that over half the initially live cells are lost in the freezing process. A comparison of the balance of the pre-freeze and post-freeze changes is presented in Table 2. The percent normal cells decreased as did the normal cells with nonaged acrosomes and the overall percentage of nonaged acrosomes. Or, freezing decreased the percent live cells and percent normal cells and increased the degree of aging noted in the ejaculates. It is interesting to note that the percentage of normal cells with aged acrosomes remained unchanged.

Table 1. Overall Means ($\bar{X}$) and Standard Deviations (S.D.) for the Spermatozoan Characteristics Studied

Characteristic	$\bar{X}$ (%)	S.D. (%)
Pre-freeze Evaluation		
Live Spermatozoa	81.5	6.4
Aged Acrosomes	34.7	10.9
Normal Spermatozoa	74.1	10.3
Normal Cells with Aged Acrosomes	21.1	8.9
Normal Cells with Nonaged Acrosomes	53.0	10.8
Abnormal Cells with Nonaged Acrosomes	11.9	6.7
Abnormal Cells with Aged Acrosomes	13.6	5.8
Nonaged Acrosomes	65.0	11.3
Post-freeze Evaluation		
Motility (O-storage time)	32.5	7.3
Motility (1 month storage time)	31.2	5.3
Aged Acrosomes	47.9	12.4
Normal Spermatozoa	59.1	15.3
Normal Cells with Aged Acrosomes	20.2	3.9
Normal Cells with Nonaged Acrosomes	38.9	14.3
Abnormal Cells with Nonaged Acrosomes	11.9	4.4
Abnormal Cells with Aged Acrosomes	27.7	13.1
Nonaged Acrosomes	50.8	14.4

Table 2. Influence of Freezing on Various Sperm Cell Characteristics

Sperm Cell Characteristic	Mean-% (243 collections)		
	Pre-freeze	Post-freeze	Change
Normal Cells	74.5	60.2	−14.3
Aged Acrosomes	34.2	48.2	+14.0
Normal Cells with Aged Acrosomes	20.8	20.5	− 0.3
Normal Cells with Nonaged Acrosomes	53.7	39.7	−14.0
Abnormal Cells with Nonaged Acrosomes	11.8	12.0	+ 0.2
Abnormal Cells with Aged Acrosomes	13.4	27.7	+14.3
Nonaged Acrosomes	65.5	50.9	−14.6

Table 3 lists the ten variables, in order of importance, that added significantly ($P<.05$) to the prediction of 60-90 day non-return rate. Progressive motility post-freeze and the morphology of cells pre-freeze are indicated to be two of the more important variables in the prediction of fertility. This is in agreement with the literature which for years has indicated motility and morphology to be important aspects of semen evaluation.

In addition to these characteristics, it is evident from Table 3 that the state of the acrosome should also be considered in semen evaluation. Five of the ten variables that add significantly to the prediction of fertility involve the acrosome. This indicates that the acrosome is too important in semen evaluation to be ignored and should play an important role in routine fertility evaluation. Table 3 also shows that certain pre-freeze characteristics contributed significantly to the prediction of fertility. The percentage of normal cells pre-freeze with aged acrosomes is a highly important measurement which does not change significantly with freezing (Table 2). The total percentage of acrosomal aging in the initial ejaculate is also important. The percentages of normal cells and live cells pre-freeze also add significantly to prediction of fertility.

It should be realized that the last three pre-freeze characteristics mentioned change appreciably during freezing and post-freeze measurements of these characteristics are also present in the list of significant components of the prediction system (Table 3).

The variables listed in Table 3 were utilized in various combinations to determine the precision with which fertility was predicted. These results are summarized in Table 4. When all ten variables were utilized, 86 percent of the bulls observed to be above or below average were

Table 3. Variables Having a Significant Effect in Prediction of Fertility*

1. Post-freeze motility ("0"—storage time).
2. Pre-freeze normal cells with aged acrosomes.
3. Pre-freeze normal cells.
4. Post-freeze motility (1 month storage time).
5. Post freeze aged acrosomes.
6. Post-freeze nonaged acrosomes.
7. Pre-freeze aged acrosomes.
8. Pre-freeze live cells.
9. Post-freeze normal cells with aged acrosomes.
10. Post-freeze normal cells.

*$P < .05$ for all variables.

computed to be so. The combinations of three, four or six variables were less precise in the prediction resulting, ranging from 74 percent to 79 percent.

Table 4 presents another way to evaluate the predictor system. The percentage of bulls where the computed fertility was within 5 percent of the observed value ranged from 71 percent to 76 percent with ten variables being somewhat superior to the other combinations. When the limit was increased, 93 percent to 95 percent of the computed values were within 7 percent of the observed fertility. The weighting factors utilized in the above combinations of variables are presently being refined and will be published in detail at a later date.

Table 4. Summary of Predictions of Fertility

Combinations of variables	Bulls classified correctly as above or below average	No. bulls where computed value was within 5% of observed	No bulls where computed value was within 7% of observed
Combination 1 (All 10 variables)	36/42 = 86%	32/42 = 76%	39/42 = 93%
Combination 2 (3 variables)	33/42 = 79%	30/42 = 71%	40/42 = 95%
Combination 3 (4 variables)	31/42 = 74%	31/42 = 74%	39/42 = 93%
Combination 4 (6 variables)	31/42 = 74%	31/42 = 74%	39/42 = 93%

Results from this study strongly indicate that the state of the acrosome is an important aid in trying to predict the potential fertility of a bull. At this time it appears that pre-freeze and post-freeze determinations of motility, morphology and state of the acrosome are all important spermatozoan characteristics in the prediction of fertility.

Literatre Cited

Bishop, M. W. H., R. C. Campbell, J. L. Hancock and A. Walton. 1954. J. Agri. Sci. 44:227.

Erb, R. E., M. E. Ehlers, L. Mikota, and E. Schwarz. 1951. Wash. Agr. Expt. Sta. Tech. Bulletin No 2.

Hancock, J. L. 1952. J. Exptl. Biol. 29:445.

Lasley, John F. and Ralph Bogart. 1943. Mo. Ag. Exp. Sta. Res. Bul. 376.

Munroe, I. B. 1961. J. Reprod. Fertil. 2:513.

Saacke, R. G. 1970. Proc. 3rd Technical on A. I. and Reprod. N.A.A.B. 17-29.

Saacke, R. G., R. R. Amann, and C. E. Marshall. 1968. J. Anim. Sci. 27:1391.

Saacke, R. G. and C. E. Marshall. 1968. J. Reprod. Fert. 16:511.

Saacke, R. G. and J. M. White. 1972. J. Anim. Sci. 35:253.

Shaffer, H. E. and J. O. Almquist. 1949. J. Dairy Sci. 32:723.

Wells, M. E. and O. A. Awa. 1970. J. Dairy Sci. 53:227.