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Correlation Between Clusterin-Positive Spermatozoa Determined by Flow Cytometry in Bull Semen and Fertility

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ABSTRACT: The objectives were to 1) develop a rapid and accurate method for detection of clusterin-positive spermatozoa (CPS) in bull semen and 2) determine the utility of incidence of CPS for prediction of fertility of bull semen in comparison to routine semen quality traits. Semen from 3 bulls was immunostained with anti-bovine clusterin antibody and with FITC-conjugated anti-rabbit IgG for method development. Clusterin-positive spermatozoa were determined by flow cytometry (FCM) and fluorescence microscopy, and results were compared by paired *t* test. There was no difference between FCM and microscopic techniques ($P = .81$). Flow cytometry was then used for determination of CPS in semen of 48 bulls with known fertility. Significant inverse relationships were found between the percentage of CPS and raw nonreturn rate ($r = -.30$), adjusted nonreturn rate ($r = -.58$), and estimated relative conception rate (ERCR; $r = -.60$). Estimated relative conception rate is potentially a very accurate method for determining fertility, and it resulted in highest correlation with CPS. An inverse relationship was observed between the percentage of CPS and prefreeze and postfreeze motility ($r = -.51$), whereas a direct relationship was found between CPS and primary, secondary, tertiary, and total sperm abnormalities ($r = .52, .77, .32$, and $.58$, respectively). The fractions of motile and abnormal spermatozoa, with the exception of tertiary abnormalities, were inversely correlated with 2 or more of the fertility estimates, but none of them showed the characteristic increase in correlation with improvement of accuracy of fertility estimate as demonstrated by CPS. We conclude that FCM is useful for objective and efficient detection of CPS in bull semen. The results suggest that the percentage of CPS in bull semen is potentially a better predictor of fertility than sperm motility or abnormal morphology.

Key words: Clusterin, semen evaluation, nonreturn rate.

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In the cattle artificial insemination (AI) industry, bull fertility is highly important economically. Several indices of fertility are used, such as services per conception, calving rate, and nonreturn rate (Salisbury et al, 1978). Before preservation and distribution for use in AI, several laboratory tests are routinely conducted in an attempt to predict the fertility of the processed semen. Such tests include but are not limited to sperm motility or viability, total sperm output, and morphology. Although these tests can detect grossly abnormal ejaculates, none of them appears to accurately predict observed differences in fertility among animals (Schenk et al, 1987; Saacke et al, 1995). Fluorescence-activated cell sorting technique (flow cytometry) facilitates determination of multiple sperm characteristics in a high number of spermatozoa, thereby increasing specificity and efficiency of semen analysis (Graham et al, 1990; Morell, 1991). Applications of flow cytometry in artificial insemination revealed correlations between sperm chromatin structure and fertility in bulls (Evenson et al, 1980; Ballachey et al, 1987, 1988; Dobrinski et al, 1994). This technique has also been used to demonstrate correlations between sperm membrane integrity, mitochondrial function and classical semen traits and fertility of bull semen. However, correlation coefficients were not significant when flow cytometer or classical semen traits were analyzed separately (Ericsson et al, 1993).

Clusterin, an acidic heterodimeric glycoprotein produced both in the testis and epididymis, is associated with spermatozoa in the ram (Tung and Fritz, 1985), rat (Sylvester et al, 1984, 1991), and bull (Howes et al, 1998; Ibrahim, 1998; Ibrahim et al, 1999). In human ejaculated semen, the heterodimeric form of clusterin was detected only on abnormal spermatozoa (O'Bryan et al, 1990, 1994). Similarly, bull and ram spermatozoa that exhibited head, midpiece, and principal piece abnormalities intensely reacted with anticlusterin antibody (Ibrahim, 1998). Such spermatozoa were trapped in glass wool–Sephadex (GWS) columns, which is an assay method for evaluation of bull semen first proposed by Graham et al (1976). Glass wool–Sephadex assay results, as measured by the rate of passage of spermatozoa through the filters, were highly correlated ($r = .93$) with the fertility of frozen

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stallion semen (Samper et al, 1991); however, less correlation was reported (r = .52) with the fertility of bull semen (Graham et al, 1980). A reason for this might be that fertility variation in AI bulls is less than in stallions and that the traditional nonreturn rate used for measuring bull fertility in the AI industry is less accurate than the pregnancy rate used for horses. Because improved fertility measurements are now available (Clay, 1987; Fetrow et al, 1990) and because evidence exists that clusterin may be a factor in Sephadex trapping of stallion spermatozoa (Samper et al, 1995), we hypothesize that the fraction of clusterin-positive spermatozoa in semen would be a better predictor of fertility of bull semen than the routinely applied semen assays. Thus, the objectives of this study were to 1) develop a method for objective and rapid determination of incidence of clusterin-positive spermatozoa in bull semen and 2) determine correlations between the incidence of clusterin-positive spermatozoa and fertility estimates currently used in the cattle AI industry (nonreturn rates and estimated relative conception rate [ERCR]) in comparison to correlations between routine semen assays results (total sperm output and the fraction of motile and morphologically abnormal spermatozoa) and the fertility estimates.

Materials and Methods

Method Development

Three straws of frozen semen selected randomly from 1 beef bull (animal 1) and 1 dairy bull (animal 2) and 3 fresh ejaculates collected from another beef bull (animal 3) were used. Frozen and fresh semen was used in this part of the study to ensure that freezing does not affect principal pattern of clusterin reactivity. For the first 2 bulls, semen was frozen in skim milk extender and packaged in 0.5-mL French straws at a sperm concentration of 60×10^6 spermatozoa/mL and was stored in the laboratory. The fresh semen was collected 3 times from a normal 18-month-old bull housed at the University beef barn. Fresh semen concentration was determined microscopically with a hemacytometer, and an aliquot of 30 x 10^6 spermatozoa was used for each experiment. Semen was stained by the indirect immunofluorescent-antibody (IFA) technique, essentially as described by Okabe et al (1990). All reagents were purchased from Sigma (St. Louis, Mo) unless mentioned otherwise. Primary antibody was a polyclonal anti-bovine clusterin antibody (anti-bCAb) generated in rabbits against purified bovine cauda epididymal fluid clusterin (Ibrahim, 1998). The secondary antibody was a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (F-0511, Sigma). The frozen semen was thawed daily in a waterbath at 37°C for 30 seconds and divided equally in three 1.5 mL microcentrifuge tubes. Ten million spermatozoa from the fresh semen were transferred into 3 tubes. The content of 1 tube from each bull's semen was stained with primary and secondary antibodies (treatment); that of the second tube was stained with preimmune rabbit serum and secondary antibody (control); and that of the third tube was left unstained. Antibody concentration, incubation time, and washing stringency were varied to determine conditions producing optimum antigen-antibody binding as observed with a fluorescence microscope. Spermatozoa were washed 3 times with 1 mL Tris HCl-buffered saline (TBS; 10 mM Tris-HCL, 150 mM NaCl, pH 7.4) by suspension and centrifugation at $4000 \times g$ for 4 minutes in a microcentrifuge. The treatment spermatozoa were incubated with anti-bCAb diluted 1:500 in 3% bovine serum albumin (BSA)/TBS for 1 hour and were washed 2 times with 0.04% TBS-Tween 20 (TBS-T) and 3 times with TBS by centrifugation and resuspension. The control spermatozoa were incubated with preimmune rabbit serum diluted 1:500 in 3% BSA/TBS and were incubated and washed exactly as the treatment. Both treatment and control sperm cells were incubated in the dark for another hour with 1:160 FITC-conjugated goat anti-rabbit IgG in 3% BSA/TBS and were washed as described above. The unstained sperm cells were incubated in 3% BSA/TBS without the antibodies and were washed as the treatment and control tubes had been. After the last wash, the sperm pellets were suspended in 2 mL TBS and filtered through 35-μm strainer cap tubes (Becton Dickinson Labware, Franklin Lakes, NJ) to remove any sperm aggregates. The tubes were protected from direct light by aluminum foil wraps.

Sperm suspensions were subjected to fluorescent cell counting in a fluorescence-activated cell-sorting machine (FACSCalibur; Becton Dickinson, Rutherford, NJ) within 20 to 30 minutes of staining. The cytometer was set principally as described by Graham et al (1990). A 485-nm argon laser line was used for excitation. Filter setup included a 515-nm long pass filter, a 590-nm dichromic beam splitter, and a 525-nm band pass for the green fluorescence. First, 50 000 cells were acquired from the tube containing unstained sperm cells. Forward scatter, side scatter, and threshold were set so that very large or very small particles were excluded from counting. The peak produced by these cells was marked as M1. Second, 50 000 cells were acquired from the control, and the fluorescence peak originating from nonspecific secondary antibody binding was determined and marked as M2. Finally, 50 000 sperm cells were acquired from the treatment tube, and the fluorescence peak appearing because of the presence of anti-bovine clusterin antibody was identified and marked as M3. The location of the markers was kept constant among the 3 cell types acquired, and the resulting template was saved and used for subsequent analysis. Data were collected and analyzed by using the CellQuest software package (Becton Dickinson, Rutherford, NJ), loaded onto a Power Mac personal computer connected to the cytometer. One parameter histogram was generated for each sample, in which the X-axis represented mean fluorescence intensity for cells passing through the channel for green fluorescence (FL1-H), and the Y-axis represented the number of sperm cells. The percentage of cells in the 3 regions marked M1, M2, and M3 featuring least fluorescence (M1) and highest fluorescence (M3) along the X-axis was calculated. Regions M1 and M2 contained sperm cells with mean fluorescence intensity of 0 to 400, whereas M3 contained sperm cells with fluorescence intensity of at least 400.

Immediately after flow cytometry, slide smears were prepared from the treatment and control sperm cells, mounted with a fluorescence antifading medium (Immuno-Fluore; ICN, Aurora,

Table 1. *Summary of fertility data, semen criteria and the percentage of clusterin-positive spermatozoa from semen of the artificial insemination bulls (n = 48)*

Variable	Mean ± SD	Range
Services	7241.10 ± 8072.12	969.00 to 43683.00
Raw nonreturn rate (%)	68.79 ± 4.03	57.50 to 74.40
Adjusted nonreturn rate (%)	65.71 ± 5.07	44.30 to 72.70
Estimated relative conception rate (%)	2.92 ± 3.46	−11.00 to 9.00
Total sperm count (10^9)	12.01 ± 5.20	4.20 to 26.19
Prefreeze motility (%)	69.93 ± 4.83	60.00 to 80.00
Postfreeze motility (%)	48.51 ± 6.65	25.00 to 60.00
Primary sperm abnormalities (%)	6.12 ± 7.70	0.00 to 40.50
Secondary sperm abnormalities (%)	3.99 ± 3.50	0.50 to 17.50
Tertiary sperm abnormalities (%)	15.29 ± 12.63	2.00 to 61.50
Total sperm abnormalities (%)	25.66 ± 19.24	4.00 to 107.50
Clusterin-positive spermatozoa (%)	4.11 ± 2.46	1.25 to 12.32

Ohio) and preserved in the dark. The proportion of clusterin-positive spermatozoa was determined within 3–5 hours after slide preparation by differential counting of 200 sperm cells with a fluorescence microscope (Labophot-2, Nikon, Melville, NY). The flow cytometric and microscopic results were compared by paired *t* test using SAS software (Cary, NC).

Relationship Between Clusterin-Positive Spermatozoa, Fertility and Conventional Measurements of Semen Quality

Frozen semen of 48 dairy bulls from an artificial insemination center (Genex/Cooperative Resource International [CRI], Shawano, Wisc) was selected so that high- and low-fertility bulls were included. Summary of fertility data and semen characteristics of these bulls are presented in Table 1. The fertility data were kept at the AI center until the flow cytometric assay for clusterin-positive spermatozoa were concluded. The fertility estimates included the following: 1) raw nonreturn rate, defined as the percentage of cows or heifers reported not having returned to service within 60 to 90 days after insemination; 2) adjusted nonreturn rate which considers the percentage of heifers serviced by the bull because heifers have slightly higher conception rate than cows; and 3) ERCR, a fertility ranking of a service sire by the Dairy Record Management System (DRMS; Raleigh, NC) relative to service sires of all herdmates that report to this organization. Routine semen evaluation results were obtained from 3 of the laboratories producing the semen. Results for 22 bulls, including total sperm output, prefreeze and postfreeze motility, and sperm abnormalities (primary, secondary, tertiary, and total) were obtained from the laboratory in Shawano, Wisconsin. Semen evaluation results for 6 bulls were obtained from the laboratory in Tiffin, Ohio; and for 20 bulls, from the laboratory in Ithaca, New York. The routine semen assay results were mostly obtained from the same ejaculate analyzed for clusterin. However, when these results were missing, the average of the nearest preceding and subsequent ejaculates was used for statistical measurements. Total sperm output for the 6 bulls housed at Tiffin was not available; nor were total sperm output or prefreeze or postfreeze motility for 7 bulls out of 20 housed at Ithaca. Sperm morphology for the 20 bulls housed at Ithaca was determined in our laboratory using Eosin/Nigrosin Morphology Staining (Lane Manufacturing, Denver, Co) and formal saline fixation. Sperm abnormalities were classified as primary, secondary, or tertiary according to severity and/or origin of malformation (Barth and Oko, 1989).

To determine the percentage of clusterin-positive spermatozoa, 1 straw from each bull was thawed at 37°C for 30 seconds. The semen was stained with anti-bovine clusterin antibody and subjected to flow cytometry as described under ''Method Development.''

After the proportion of clusterin-positive spermatozoa in semen of each bull had been determined, the results were sent to the AI center, where they were matched with bull fertility and semen quality results and sent back for statistical analysis. The relationships between the proportion of clusterin-positive spermatozoa, fertility estimates, and semen characteristics, as well as those between semen characteristics and fertility estimates were determined by a simple correlation procedure using SAS software. Correlation coefficients with $P \leq .05$ were considered significant.

Results

Flow Cytometry for Determination of Clusterin-Positive Spermatozoa in Bull Semen

Three experiments were conducted for the determination of the percentage of clusterin-positive spermatozoa (CPS) in semen from 3 bulls using flow cytometery and fluorescence microscopy techniques. In the 3 animals used, reactivity with anti-bovine clusterin antibody was limited to abnormal spermatozoa. The means plus or minus the standard deviations of the percentage of CPS in semen from these bulls are shown in Table 2. Comparison of these means by the *t* test revealed no significant difference either between the techniques or among the animals ($P = .81$). The flow cytometer output, expressed as number and fluorescence intensity of unstained, control (treated with preimmune rabbit serum and FITC-conjugated anti-rabbit IgG), and treatment sperm cells (stained with anti-bCAb

Table 2. *Average percentage of clusterin-positive spermatozoa in bull semen determined by flow cytometry and fluorescence microscopy (n = 3)*

Animal	Flow cytometry Mean ± SD	Fluorescence microscopy Mean ± SD
1	9.9* ± 2.4	10.1* ± 3.2
2	6.7* ± 3.8	7.3* ± 1.5
3	10.7* ± 1.5	10.3* ± 3.5

* Statistically similar ($P = .81$).

and FITC-conjugated anti-rabbit IgG) for semen of bull 1 used during the procedures described in ''Method Development'' is shown in Figure 1A through C, respectively. The mean fluorescence intensity (mean) of 98.88% of the unstained sperm cells passing through the green fluorescence (FL1-H) channel was 3.01 (region M1, Figure 1A). There were no sperm cells within regions M2 and M3 in the unstained sperm population (Figure 1A). A shift in the mean fluorescence intensity to 27.96 was observed in the M1 region cells from the control tube, possibly due to nonspecific binding of the second antibody to the head and principal piece of sperm tails that created subpeaks (a) and (b) (Figure 1B). A small population of cells showed a third peak of fluorescence corresponding to the M2 region, with a mean fluorescence intensity of 133.64 (Figure 1B). The total percentage of sperm cells falling within the M1 and M2 regions (99.8%) was classified as clusterin negative. There were 0.29% sperm cells in the control sample with fluorescence intensity of at least 400 (M3, Figure 1B). The output for the anti-bCAb-treated cells revealed that 12.59% were intensely fluorescent (M3, Figure 1C). These were clusterin-positive spermatozoa. They appeared bright green after excitation with ultraviolet light of 450 to 490 nm wavelength using a fluorescence microscope (Figure $2A_1$). Similar numbers of clusterin-positive cells (13.4%) were found by differential counting with fluorescence microscopy of a slide smear prepared immediately after flow cytometry. The population of spermatozoa appearing to the left of the M3 region that represented 87.53% of the total cells were the clusterin-negative cells and were invisible with fluorescence microscopy (Figure $2A_1$).

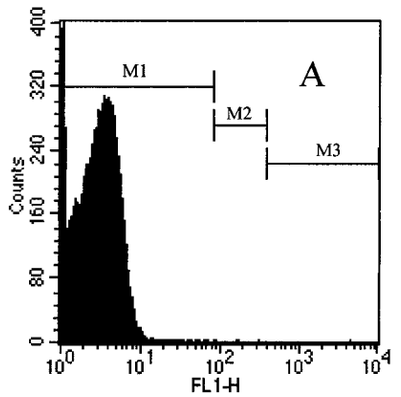
Correlations Among CPS, Fertility, and Semen Quality

Average percentage of CPS detected in bull semen from the AI center (n = 48) was 4.11, with a range of 1.25 to 12.32 and a standard deviation of 2.46. With the exception of total sperm output, all semen parameters analyzed demonstrated significant correlations ($P \leq .05$) with CPS (Table 3). Negative significant correlations were found between the proportion of CPS and raw and adjusted nonreturn rates and ERCR. Similarly, an inverse relationship was observed between CPS and prefreeze or postfreeze motility, whereas a direct relationship was seen between CPS and primary, secondary, tertiary, and total abnormal spermatozoa. The fertility indices correlated positively with each other, but the correlation between ERCR and adjusted nonreturn rate was higher ($r = .53$) than with raw nonreturn rate ($r = .35$). Like CPS, prefreeze motility showed direct relationship to fertility measurements but did not show the characteristic increase in correlation coefficient with improvement of the fertility estimate, as was observed with CPS. Postfreeze motility correlated directly with nonreturn rate but not with ERCR. Primary and total sperm abnormalities correlated directly with nonreturn rate but not with ERCR. However, secondary abnormalities correlated with all 3 fertility estimates. Tertiary sperm abnormalities exhibited no relationship to any of the fertility measurements.

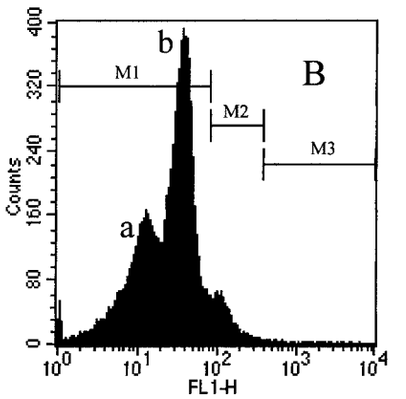
Discussion

We describe that flow cytometry is useful for objective and efficient detection of clusterin-positive spermatozoa (CPS) in bull semen. Because the markers gating regions M1–M3 were saved permanently in an analysis template for the entire study, variation in results due to human error in counting fluorescent cells with the fluorescence microscope was overcome. Hence, flow cytometry provides more consistent results in determination of the proportion of clusterin-positive spermatozoa in bull semen than does fluorescence microscopy. Likewise, scanning of up to 50 000 sperm cells increases objectivity and repeatability of measurements. One of the problems in estimating fluorescent cells by fluorescence microscopy is fluorescence fading within a short period of ultraviolet light exposure. Flow cytometry bypasses this problem by the capacity for differential counting and classifying of tens of thousands of cells within seconds. Flow cytometry also minimizes the time and labor needed for preparation and counting of cells on dry-slide smears. The results for detection of CPS with flow cytometry were available at least 5 hours before dry slide results could be obtained.

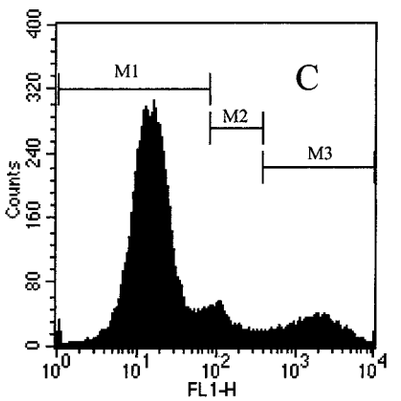
Only sperm cells falling within region M3, thus having a mean fluorescence intensity of at least 400, were considered clusterin positive. Detection by flow cytometry of a minor population of sperm cells in the M2 region when control (Figure 1B) or treatment (Figure 1C) samples were acquired could be attributed to the high sensitivity of the machine. It is possible that this peak of fluorescence originates from nonspecific binding of the secondary antibody to the sperm midpiece. Because this fluorescence was infrequently recognized and barely visible when observed with the fluorescence microscope, M2 region cells were considered clusterin negative. Subpeaks a and b (Figure 1B) may be a product of the faint nonspe-



Marker	Events	% Gated	Mean
All	50000	100.00	3.09
M1	49442	98.88	3.01
M2	5	0.01	172.83
M3	1	0.00	4104.70



Marker	Events	% Gated	Mean
All	50000	100.00	38.00
M1	46480	92.96	27.96
M2	3419	6.84	133.64
M3	143	0.29	1044.43



Marker	Events	% Gated	Mean
All	50000	100.00	287.30
M1	39924	79.85	19.00
M2	3840	7.68	165.27
M3	6295	12.59	2062.50

Figure 1. Two-dimensional histogram representation of the flow cytometer output for determination of clusterin-positive spermatozoa in semen of bull 1 used during method development. Clusterin was localized on bull spermatozoa as described in the ''Materials and Methods'' section. **(A)** Unstained sperm cells. **(B)** Control spermatozoa (treated with preimmune rabbit serum and fluorescein isothiocyanate–conjugated anti-rabbit IgG). **(C)** Anti-bovine clusterin antibody and FITC-conjugated anti-rabbit IgG-treated spermatozoa. Cells located within M1 and M2 regions are clusterin negative. Region M3 contains clusterin-positive cells.

cific staining of the head and principal piece, which was observed less often under the microscope than midpiece staining. All nonspecific reactivities were rarely discernible and so weak that they could not be recorded by photomicrography.

Nonreturn rates are estimates of biological measures for reproductive efficiency, such as conception rate and calving rate, which are more reliable than nonreturn rate itself for the evaluation of bull fertility (Koops et al, 1995). Adjusted nonreturn rate is more accurate than raw nonreturn rate because it accounts for the number of heifers inseminated; and recently, a further improved measurement of fertility, ERCR, has been introduced by the DRMS (Clay, 1987; Fetrow et al, 1990). ERCR is cal-

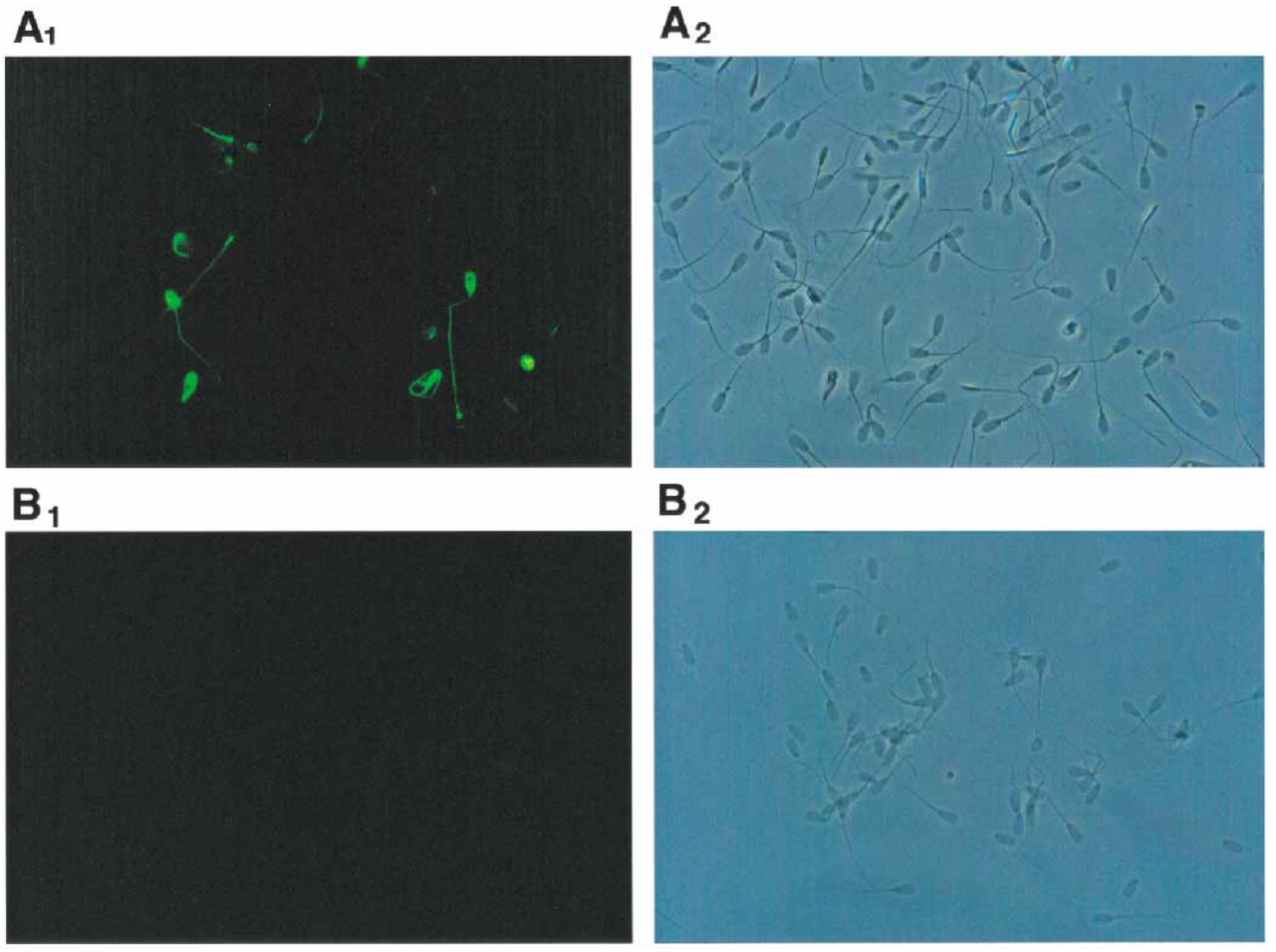


Figure 2. Indirect fluorescence of clusterin on bull spermatozoa. Clusterin was localized on spermatozoa as described in the ''Materials and Methods'' section. **(A_1)** Treatment. Fluorophotograph of spermatozoa stained with anti-bovine clusterin antibody and FITC-conjugated anti-rabbit IgG. **(B_1)** Control. Fluorophotograph of spermatozoa stained with preimmune rabbit serum and FITC-conjugated anti-rabbit IgG. **(A_2, B_2)** Phase contrast–photographs of corresponding fields on **(A_1)** and **(B_1)**, respectively. A small proportion of spermatozoa were clusterin positive and were all morphologically abnormal **(A_1)**.

culated from all AI services performed within the most recent 3 years for all Holstein and Jersey bulls in Dairy Herd Improvement (DHI) herds of all states that report to DRMS. Because it is calculated out of many services from many different herds, ERCR is believed to be a highly accurate measurement for identifying high- and low-fertility bulls. In the current study, significant correlations between CPS and raw nonreturn rate, adjusted

Table 3. *Correlations between the proportion of clusterin-positive spermatozoa in bull semen (CPS) and fertility (raw nonreturn rate [RNR], adjusted nonreturn rate [ANR], and estimated relative conception rate [ERCR]), between CPS and semen characteristics (prefreeze motility [PREFMOT], postfreeze motility [PSTFMOT], primary sperm abnormalities [1°ABN], secondary abnormalities [2°ABN], tertiary abnormalities [3°ABN], and total abnormal spermatozoa [TOTABN]) and between semen characteristics and fertility.*

Variable	CPS	PREFMOT	PSTFMOT	1°ABN	2°ABN	3°ABN	TOTABN
CPS	. . .	−0.51***	−0.51***	0.52***	0.77***	0.32*	0.58***
RNR	−0.30*	0.36*	0.44**	−0.41**	−0.36*	0.23†	−0.39**
ANR	−0.58***	0.50***	0.39*	−0.51***	−0.60***	−0.24†	−0.49***
ERCR	−0.60***	0.36*	0.30†	−0.18†	−0.39**	0.00†	−0.19†

* .01 ≤ *P* ≤ .05.
** .001 ≤ *P* ≤ .01.
*** .0001 ≤ *P* ≤ .001.
† Not significant.

nonreturn rate, and ERCR were found. Furthermore, the relationship between CPS and the fertility estimates exhibited a linear pattern of increase with improvement of accuracy of the fertility determinant from raw nonreturn rate to adjusted nonreturn rate and ERCR. Such pattern was not present for any of the semen evaluation criteria examined except CPS, suggesting that CPS in semen is better related to fertility than abnormal sperm morphology or motility. Microscopic observation of the anti-bCAb–stained slides under high power (1000×) revealed that all clusterin-positive spermatozoa were abnormal but that not all abnormal spermatozoa were clusterin positive. This indicates that the use of CPS for semen evaluation is not identical to semen evaluation for morphology.

The reason for clusterin accumulation on abnormal spermatozoa is unknown. However, clusterin has been associated with cell damage in several disease conditions (Rosenberg and Silkensen, 1995; Kock-Brandt and Morgans, 1996). Overaccumulation of clusterin was noticed on rat germ cells because of treatment with methoxyacetic acid (Clark et al, 1997), and augmentation of its mRNA production was observed in rat and mouse Sertoli cells exposed to high temperature in vitro (Clark and Griswold, 1997). We recently observed that localized testicular hyperthermia inflicted by scrotal insulation has increased dramatically the percentage of clusterin-positive spermatozoa in ram semen in association with increase in abnormal spermatozoa from day 15 until day 60 after initiation of insulation (Ibrahim, 1998). Thus, the accumulation of clusterin on abnormal spermatozoa in normal bull semen may be an indication of unfavorable testicular conditions or individual germ cell aberrations that might have an effect on fertility but are undetectable by current routinely used laboratory tests.

Glass wool–Sephadex filtration eliminated dead, immotile, and abnormal spermatozoa from bull semen and improved nonreturn rate of low-fertility bulls from 61% to 67% (Graham and Graham, 1990). It was also demonstrated, after induction of testicular degeneration by scrotal insulation, that more abnormal spermatozoa were trapped in the GWS filters than normal sperm forms (Crabo et al, 1992). Sperm membrane clusterin has been implicated in the process of trapping of abnormal equine spermatozoa by the GWS filters (Samper et al, 1995). The present study shows that the incidence of CPS and morphologically abnormal spermatozoa were correlated. It is known that correlations between fertility and semen traits have been improved when combinations of semen traits have been considered (Ericsson et al, 1993). It is possible that multiple unfavorable traits on the sperm shared clusterin as a common biochemical feature, making it a potentially better candidate for prediction of fertility than, for example, sperm morphology or motility.

In conclusion, flow cytometric determination of the incidence of clusterin-positive spermatozoa is a rapid and objective method for bull semen assay. The resulting proportion of clusterin-positive spermatozoa is better correlated to bull fertility than traditional semen assays and has potential to be utilized as a good predictor of fertility.

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