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Monoclonal Antibody to Human Fertilization Antigen-1 (FA-1) Inhibits Bovine Fertilization In Vitro: Application in Immunocontraception[1]

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ABSTRACT

A monoclonal antibody (mAb) to the human sperm plasma membrane protein, fertilization antigen-1 (FA-1), was tested for its reactivity with bovine spermatozoa and its effects on bovine fertilization in vitro. Western blot analysis revealed that the FA-1 mAb reacted with proteins of similar molecular mass (53 ± 2 kDa) in human and bovine sodium deoxycholate (DOC)-solubilized sperm extracts. Indirect immunofluorescence, using epifluorescence microscopy and laser scanning confocal microscopy, revealed that the FA-1 antigen is present in the post-acrosomal region of bovine spermatozoa, which is similar to human FA-1 localization. In bovine in vitro fertilization (IVF) trials, using oocytes obtained from slaughterhouse ovaries, addition of 20, 40, or 80 μg/ml of FA-1 mAb to the IVF medium resulted in a linear decrease in the fertilization rate from 86.3% in the controls to 54.6%, 21.6%, and 1.8% in the respective experimental groups ($p < 0.01$). There was no inhibitory effect ($p > 0.10$) of the FA-1 mAb on percent sperm motility or other motility characteristics tested, suggesting that human FA-1 mAb inhibits bovine sperm cell function at some point after capacitation. In conclusion, the evolutionarily conserved antigen FA-1 has a molecular identity in bovine sperm similar to that in human sperm, and mAb to human sperm FA-1 inhibits fertilization of bovine oocytes. These results indicate that FA-1 is a promising candidate for the development of a contraceptive vaccine. The research also suggests that bovine species could be used as a model for investigating the use of FA-1 as an immunovaccine in ruminants.

INTRODUCTION

The development of contraceptive vaccines using sperm antigens is a promising approach to regulating fertility in humans and other species. By utilizing hybridoma technology, researchers have produced monoclonal antibodies (mAbs) against human sperm-specific antigens and have demonstrated that several of these antigens are involved in fertilization [1]. Monoclonal antibody probes have also been used to establish that several fertilization-related antigens present on human spermatozoa are evolutionarily conserved among various mammalian species [2, 3]. A mAb generated against the human acrosomal antigen SP-10, for example, cross-reacts with primate and pig acrosomal antigens [4]. Another mAb, AG7, generated against a human sperm plasma membrane antigen, has demonstrated cross-reactivity with species such as sheep, goats, rabbits, mice, and cynomolgus monkeys [5].

Some of these cross-reactive human sperm antigen mAbs have been utilized in fertility trials in laboratory animals to test the ability of the antibodies to inhibit fertilization. For example, a mAb developed against a human sperm-specific glycoprotein, fertilization antigen-1 (FA-1), was shown to completely block binding and penetration of zona-free hamster ova by human sperm as well as to significantly inhibit sperm penetration in in vitro fertilization (IVF) trials in mice [6, 7]. The FA-1 antibodies also blocked fertilization in female rabbits artificially inseminated with sperm that had been treated with FA-1 mAb [8].

Society's attitude toward controlling nondomestic animal populations in the wild is currently changing. Traditional methods of animal control such as hunting, trapping, poisoning, and relocation are becoming controversial and are not used as often as they once were. This has led to uncontrolled population increases of some species of animals throughout the world [9]. Ongoing research at our laboratory uses oocytes obtained from slaughterhouse cattle and sheep to study various aspects of oocyte maturation, fertilization, and early embryonic development. This system seems well suited for studying the effects of human sperm mAbs on fertilization in these ruminants. In this report, we present data indicating that a bovine sperm antigen, which reacts with human FA-1 mAb, is present in the same region of bovine spermatozoa as in human sperm cells and that the FA-1 mAb significantly inhibits fertilization of bovine oocytes in IVF trials. Species cross-reactivity of the FA-1 mAb with bovine sperm makes this antigen a good candidate for the development of a contraceptive vaccine for ruminants.

MATERIALS AND METHODS

Antibody Preparation

Ascites fluid was raised in pristine-sensitized BALB/c female mice (Jackson Laboratories, Bar Harbor, ME) against

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the FA-1 hybridomas, and the mAb was isolated by precipitation with 18% Na_2SO_4, and then diethylaminoethyl cellulose chromatography, followed by immunopurification on FA-1-coupled Sepharose-4B (activated by CNBr), as described elsewhere [7]. The affinity-purified mAb was of the IgG_{2a} subclass, and affinity-purified antibody of the same subclass from control myeloma ascites fluid (mouse IgG_{2a} (K), Cappel Laboratories, Organon Teknika Corporation, Durham, NC) was used as a control antibody to determine the nonspecific effects of the murine immunoglobulins in all assays.

Collection of Sperm Cells and Testicular Tissue

Human spermatozoa were collected from healthy fertile donors. Semen was liquefied for 30–60 min and analyzed for volume, sperm concentration, and percentage of progressive motility. Only human semen samples having > 50% motility, > 40 × 10^6 sperm/ml, and a progressive motility of > 3 (on a scale of 0 to +5) were used in this study. Fresh sperm samples were suspended in PBS containing 5% fetal bovine serum until use. Frozen sperm samples were stored in cryotubes (Nunc, Kamstrup, Denmark) in an egg yolk-citrate-glycerol (7%) extender, and samples were thawed at room temperature for 15 min before use [10]. Bovine spermatozoa were collected from mature fertile donors by electroejaculation. Only bovine semen samples having > 50% motility, > 400 × 10^6 sperm/ml, and a progressive motility > 3 were used. Samples were frozen in 0.5-cc straws in the same extender as human samples and thawed in a 39°C water bath for 1 min. Bovine testes were obtained from an abattoir, minced, and maintained in vials in liquid nitrogen until needed.

Western Blot Procedure

Sperm and testis samples homogenized in Tris-HCl were solubilized with 15 mM sodium deoxycholate (DOC) containing 1 mM PMSF and 5 mM soybean trypsin inhibitor for 30 min at 5°C [7]. The samples were then dialyzed (membrane pore size 3500) against 10 mM Tris-HCL for 48 h with three changes of dialysis buffer. Protein concentrations were determined [11], and aliquots of 150 μg of protein were lyophilized overnight. The samples were resuspended in 20 μl ddH_20, and 1 μl of 5% polyethylenimine (Sigma, St. Louis, MO) was added to each sample to precipitate DNA, which was then removed by centrifugation at 10 000 rpm for 5 min at 5°C. The protein preparations were mixed with sample buffer containing 1% SDS, 0.03 M Tris (pH 6.8), 0.5% glycerol, 0.25% beta-mercaptoethanol, and 0.005% bromophenol blue dye, and then boiled for 5 min and separated under reducing conditions on one-dimensional 10% slab SDS-PAGE minigels (Mini-Protean II; Bio-Rad, Bedford, MA; [12]). Proteins were electroblotted onto nitrocellulose membranes [13], and antigens were localized by incubating the milk-blocked nitrocellulose membrane with either FA-1 mAb or a nonspecific IgG at a dilution of 1:100 overnight at room temperature. After a second 45-min block, the blot was incubated with goat anti-mouse IgG alkaline phosphatase-conjugate (Sigma) for 2 h at room temperature. After each step in the Western blot procedure, the membrane was washed with a Tris-saline buffer containing 0.1% Triton X-100 (Sigma). Bands were visualized by use of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as a substrate [14].

Indirect Immunofluorescence Technique (IFT)

Fresh samples of bovine semen were washed three times in PBS and allowed to air-dry on methanol-cleaned slides. The slide preparations were fixed in methanol for 30 min at room temperature and washed with PBS. Nonspecific binding sites were blocked by use of PBS containing 5% BSA for 45 min and washed again with PBS. The preparations were placed in a humidified air chamber and incubated with either FA-1 mAb or nonspecific IgG overnight at 5°C at a dilution of 1:30. The primary antibody binding sites were localized by incubating the samples with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulins (Sigma) at a dilution of 1:50 for 2 h at room temperature [15]. The slides were washed gently for 5 min, and a glycerol mounting medium containing an anti-fade reagent (Slow Fade; Molecular Probes, Eugene, OR) was used to preserve the fluorescence. Sperm preparations were examined for fluorescence by means of an epifluorescence microscope equipped with a mercury lamp using a 490-nm excitation filter and a 525-nm barrier filter.

Analysis of Antigen Localization Using Scanning Confocal Microscopy

Sperm samples were prepared in the same manner as for IFT. Antigen localization was determined by laser scanning confocal microscopy using a Meridian ACAS 570 work station. A microscopic field containing spermatozoa was selected for qualitative fluorescence analysis. The inverted epifluorescence microscope objective focused an argon laser beam (excitation wavelength 488 nm) on the field and scanned the spermatozoa. For image collection, the laser excited fluorescence in a two-dimensional raster pattern, and emission was detected without a barrier filter [16].

IVF Trials

Ovaries were collected from cows at a local abattoir and were transported to the laboratory within 3–4 h in a physiological saline solution maintained at 37°C. Cumulus-oocyte complexes (COCs) were aspirated from follicles into a 50-ml conical tube by use of negative pressure (100 mm Hg) generated by a vacuum pump. Oocytes surrounded by at least three layers of cumulus cells and having a normal cytoplasmic morphology were selected, washed, and placed into 250 μl of Medium 199 containing 10% fetal calf serum,

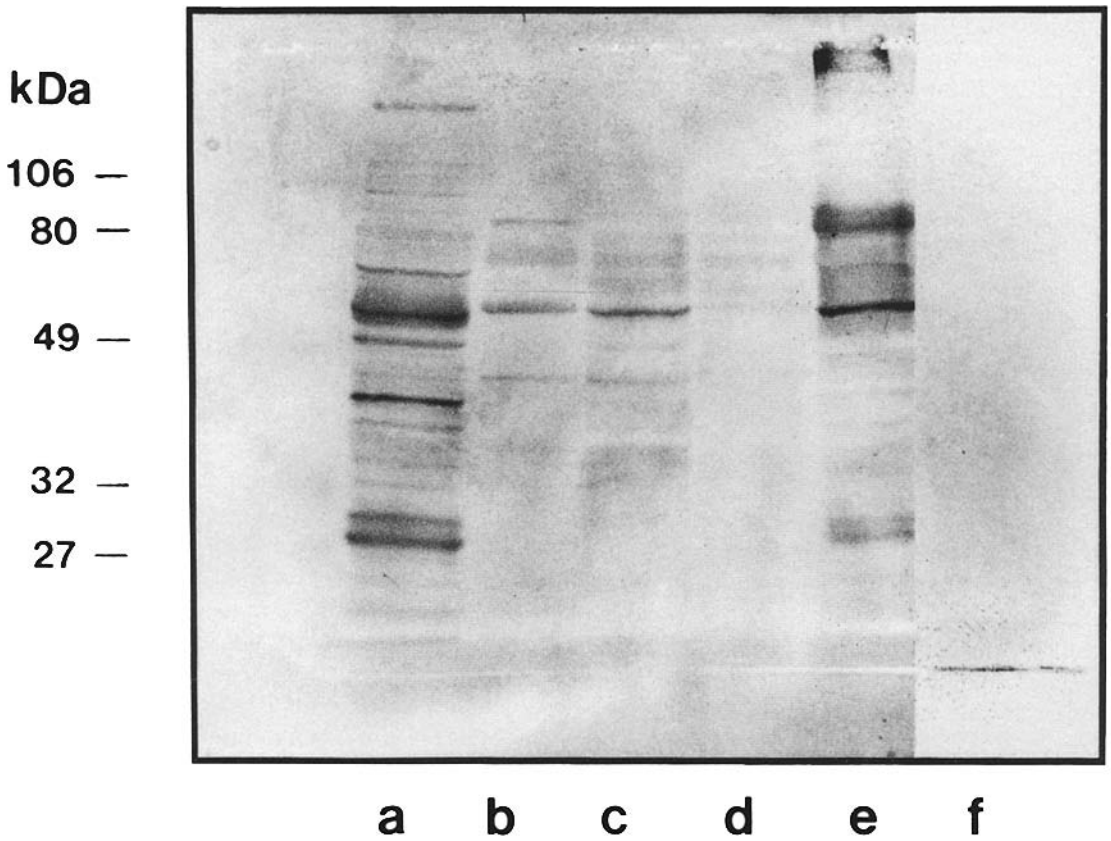

FIG. 1. Reaction of FA-1 mAb with spermatozoal antigens on Western blot of DOC-solubilized sperm extracts. FA-1 mAb reacted with a protein of 53 ± 2 kDa in human fresh (lane e) and frozen-thawed samples (lane d) and to a protein of similar size in bovine fresh (lane c), frozen-thawed (lane b), and testis samples (lane a). FA-1 mAb also reacted with a 28 ± 3-kDa protein in human fresh sperm and bovine testis samples and a 79 ± 2-kDa protein in the human fresh sperm sample, possibly representing the monomeric and trimeric forms of the FA-1 protein. The nonspecific IgG did not demonstrate any specific binding to the bovine frozen-thawed sperm sample (lane f) or any other sample (not shown).

5 μg FSH (Nobl Lab, Sioux Center, IA), and 5 μg LH (Nobl Lab). A maximum of 50 COCs per nunc well were incubated at 39°C in a humidified atmosphere containing 5% CO_2 for 24 h.

After maturation, COCs were washed three times in Tyrode's Lactate HEPES and allotted randomly into five treatment groups (with a maximum of 50 COCs per nunc well). All groups contained 450 μl of fertilization medium containing 4 μg of heparin (for capacitation) and 20 μl of PHE (hypotaurine—1 mM, epinephrine—2 mM, penicillamine—0.25 M). Treatment groups were as follows: FA-1 mAb was added to three groups at concentrations of 20, 40, and 80 μg per ml; nonspecific IgG was added to the fourth group at a concentration of 80 μg/ml; and no immunoglobulins were added to the fifth group. Frozen-thawed bovine sperm were centrifuged through a Percoll gradient, and the motile fraction was added to the wells at a concentration of 1×10^6 sperm per well. The COCs were incubated with the sperm in fertilization medium at 39°C in 5% CO_2 for 24 h [17].

To determine fertilization rates, the vortexed oocytes were fixed 24 h after fertilization with acetic-alcohol (1:3) for at least 24 h, then stained with a 1% aceto-orcein solution, and observed for pronuclear development under a dissecting microscope.

Sperm Motility Analysis

Three treatment groups, control, 80 μg/ml nonspecific IgG, and 80 μg/ml FA-1 mAb, were evaluated for their effects on sperm motion parameters using a computerized sperm analyzer (HTM-2030 Motility Analyzer; Hamilton Thorn, Beverly, MA). Sperm were incubated in IVF medium (devoid of oocytes) in the same manner as described above. Twenty-microliter aliquots of sperm suspensions were removed from the samples for analysis immediately after preparation (0 h) and after 6 h incubation at 39°C and 5% CO_2. The samples were placed in a 20-μm-deep glass semen analysis slide (Micro Cell; Norwell Technologies, Roswell, GA) and inserted into the motility analyzer. At least 100 spermatozoa per sample were evaluated in > 20 microscopic fields with 1–5 sperm/field, and the following parameters of sperm motion were measured: % motility, mean lateral head displacement (microns), progressive motility (%), and curvilinear velocity (microns/sec). The definitions of these parameters have been discussed previously [18].

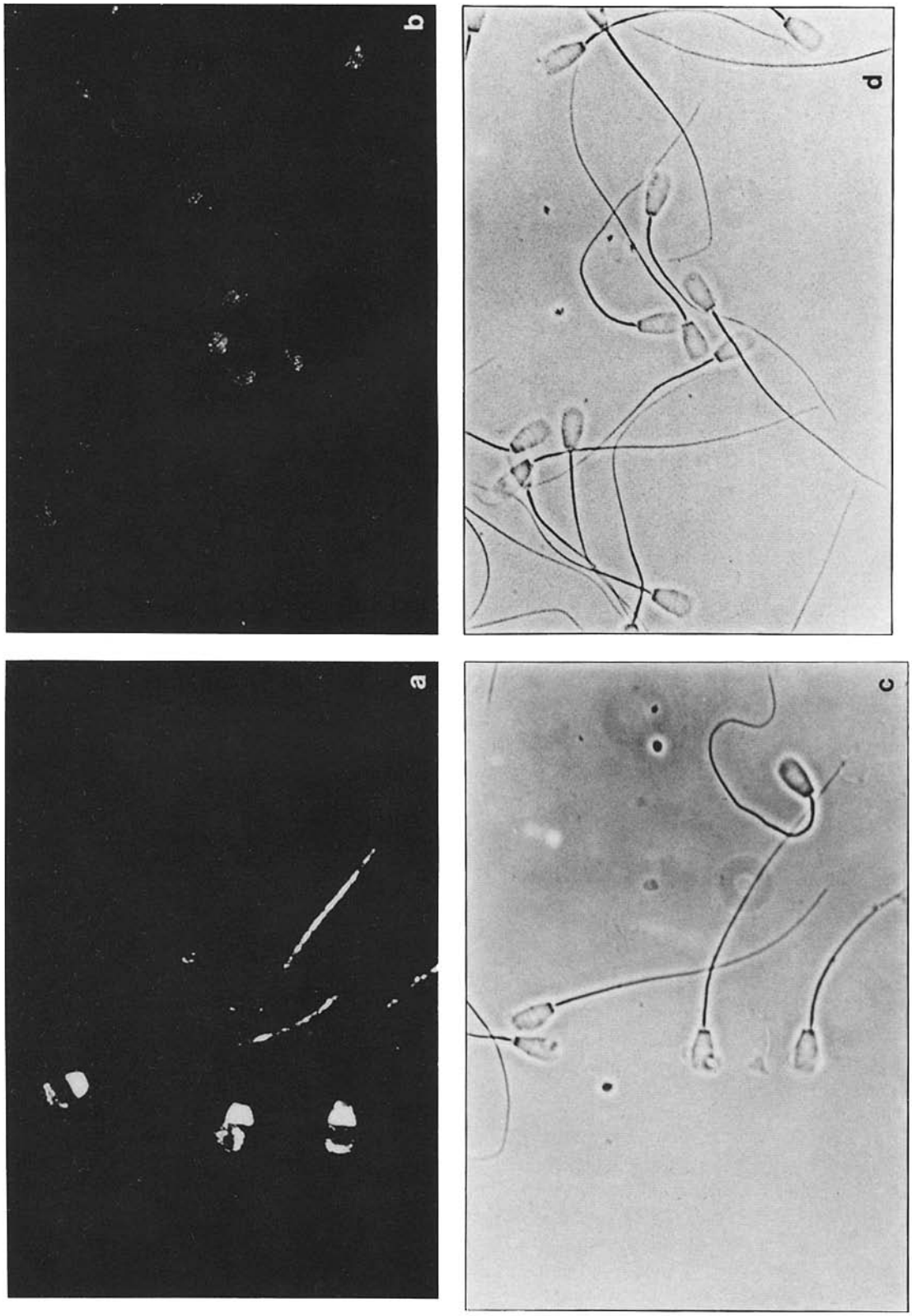

FIG. 2. Epifluorescence photomicrographs indicating the indirect immunofluorescent reaction patterns of the FA-1 mAb to bovine spermatozoa. a) The human FA-1 mAb was bound primarily to the post-acrosomal region, the tail, and to a lesser extent the acrosomal region of methanol-fixed bovine spermatozoa. b) The bovine spermatozoa incubated with nonspecific IgG showed no fluorescence to faint fluorescence patterns in the acrosomal region of the sperm head. c and d) Phase contrast photomicrographs for a and b included for comparison. ×600.

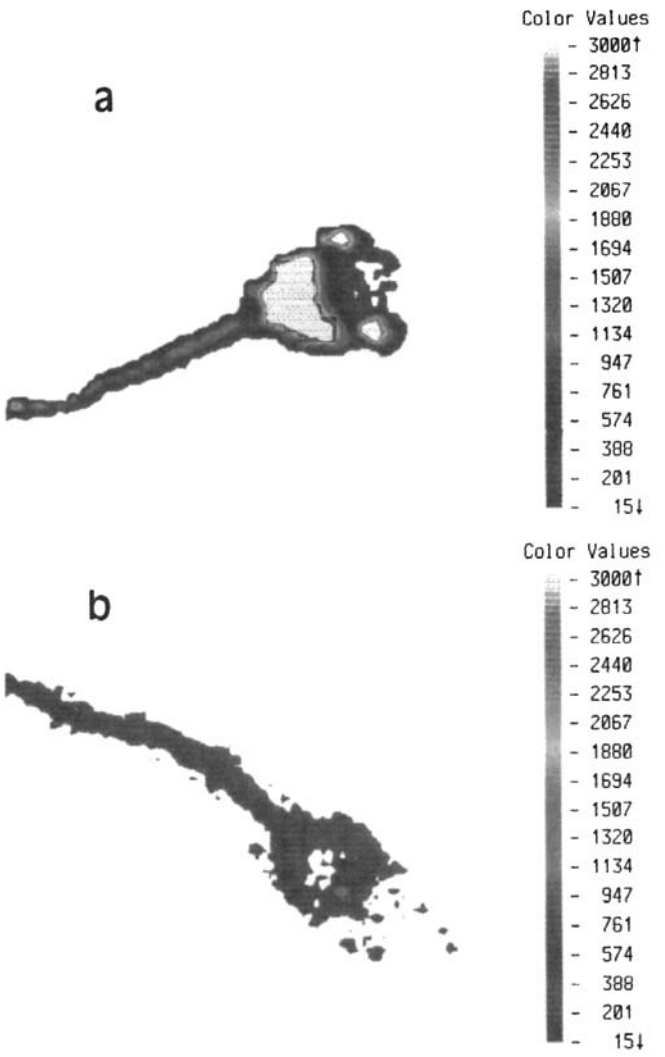

FIG. 3. Analysis of FA-1 mAb binding patterns in bovine sperm using laser scanning confocal microscopy. A microscopic field containing methanol-fixed bovine sperm was selected for qualitative fluorescence analysis, and the inverted epi-fluorescent microscope focused an argon laser beam (excitation wavelength 488 nm) on the field and scanned the spermatozoa. For image collection, the laser excited fluorescence in a two-dimensional raster pattern and emission was detected without a barrier filter. a) Digitized images of FA-1 mAb fluorescence patterns revealed that the area of greatest fluorescence intensity (as indicated by the lighter areas within the sperm cells) was in the post-acrosomal region of the sperm head as well as in the midpiece and anterior regions of the tail. b) The nonspecific IgG spermatozoa demonstrated negligible fluorescence values upon analysis.

Statistical Analysis

All quantitative data were subjected to least-squares analyses of variance using the General Linear Models procedures of the Statistical Analysis System [19]. The overall model detected no effects of replication on the dependent variables ($p > 0.10$). For subsequent analyses, replication was deleted from the model. Preplanned orthogonal contrasts of time and treatment and the time-treatment interaction were included in analyses of sperm motility parameters. All quantitative data are least-squares means ± SEM.

RESULTS

Detection of FA-1 in Bovine Spermatozoa

Analysis by Western Blot. FA-1 mAb reacted with protein bands of 53 ± 2 kDa in samples of human fresh (Fig. 1, lane e) and frozen-thawed sperm (Fig. 1, lane d). The lane containing the frozen-thawed sample had less protein, when compared to the other samples by silver staining; therefore, the banding pattern appears lighter. The mAb also reacted to protein bands of similar molecular size in samples of bovine fresh (Fig. 1, lane c) and frozen-thawed (Fig. 1, lane b) sperm as well as of bovine testis (Fig. 1, lane a) sample. The mAb also reacted with protein bands of 28 ± 3 and 79 ± 2 kDa in the human fresh samples and 28 ± 3 and 40 ± 3 kDa in the bovine testis sample. A number of other protein bands in the samples were also identified by the FA-1 mAb. The nonspecific IgG did not demonstrate specific binding to the bovine frozen-thawed sample (Fig. 1, lane f) or any other sample (data not shown). Fresh and frozen-thawed human and bovine samples were used for Western blots because it was initially hypothesized that the freeze-thaw procedure might decrease the affinity of FA-1 mAb for the antigen.

Analysis by IFT. Immunofluorescence microscopy showed that of the fresh bovine sperm demonstrating fluorescence (approximately 65%), the mAb to FA-1 was bound primarily to the post-acrosomal region, the tail, and to a lesser extent the acrosomal region of methanol-fixed bovine spermatozoa (Fig. 2a). The bovine spermatozoa incubated with nonspecific IgG showed no fluorescence to faint fluorescence patterns in the acrosomal region of the sperm head (Fig. 2b).

Analysis of sperm using scanning confocal microscopy. Digitized images of FA-1 mAb fluorescence patterns in bovine spermatozoa revealed that the area of greatest fluorescence intensity (as indicated by the lighter areas within the sperm cells) was in the post-acrosomal region of the sperm head. The analysis also revealed that FA-1 mAb bound to a lesser degree to the midpiece and anterior regions of the sperm tail (Fig. 3a). The nonspecific IgG spermatozoa demonstrated negligible fluorescence values under scanning confocal analysis (Fig. 3b). For the present study, the color images produced by the ACAS work station were converted to monochromatic images in an effort to reduce costs.

Effect of FA-1 mAb on Bovine IVF Trials

Given the observation that mAb to human FA-1 reacted with bovine sperm, as demonstrated by IFT and Western blotting procedures, we investigated whether this mAb had any effect on the ability of bovine spermatozoa to fertilize bovine oocytes in vitro. A total of 542 oocytes were used in four independent trials, with each treatment group containing at least 30 oocytes per replicate (in one trial, the nonspecific IgG group was excluded because of contamination). Two control groups were used, one containing 80

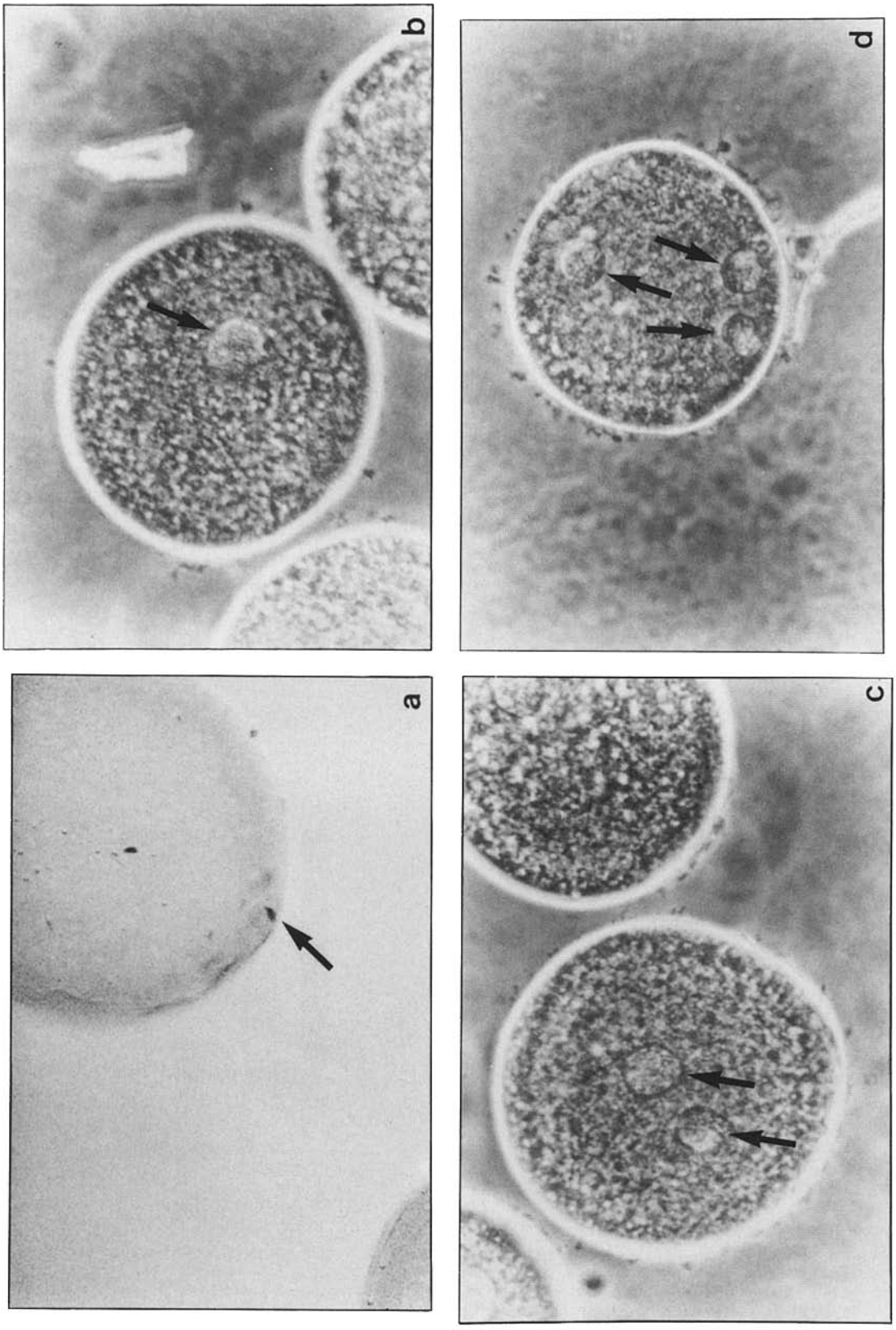

FIG. 4. Phase contrast images of aceto-orcein-stained bovine oocytes. In IVF trials to study effect of human FA-1 mAb on fertilization rates in cattle, oocytes were considered "unfertilized" if they had a) a metaphase II plate or b) only one pronucleus and "fertilized" if they had c) two pronuclei or d) were polyspermic.

TABLE 1. Effect of FA-1 mAb on fertilization rates of bovine oocytes*

Antibody	Ab. Conc. (μg/ml)	Unfertilized		Fertilized		Total number oocytes fert.	Total number oocytes	Percent fertilization**
		Metaphase II plate	1 Pronucleus	2 Pronuclei	Polyspermy			
Control (n = 4)	0	6	10	74	21	95	114	83.3% (95/114)[a]
Non-specific IgG (n = 3)	80	10	0	66	6	72	83	86.7% (72/83)[a]
FA-1 mAb (n = 4)	20	18	33	57	10	67	124	54.0% (67/124)[b]
FA-1 mAb (n = 4)	40	67	20	24	0	24	111	21.6% (24/111)[c]
FA-1 mAb (n = 4)	80	97	11	2	0	2	110	1.8% (2/110)[d]

*Bovine IVF procedure is described in *Materials and Methods.*

**Values for percent fertilization are least-squares means. SEM = 1.89

[a-d]Values followed by different letters are different ($p < 0.01$).

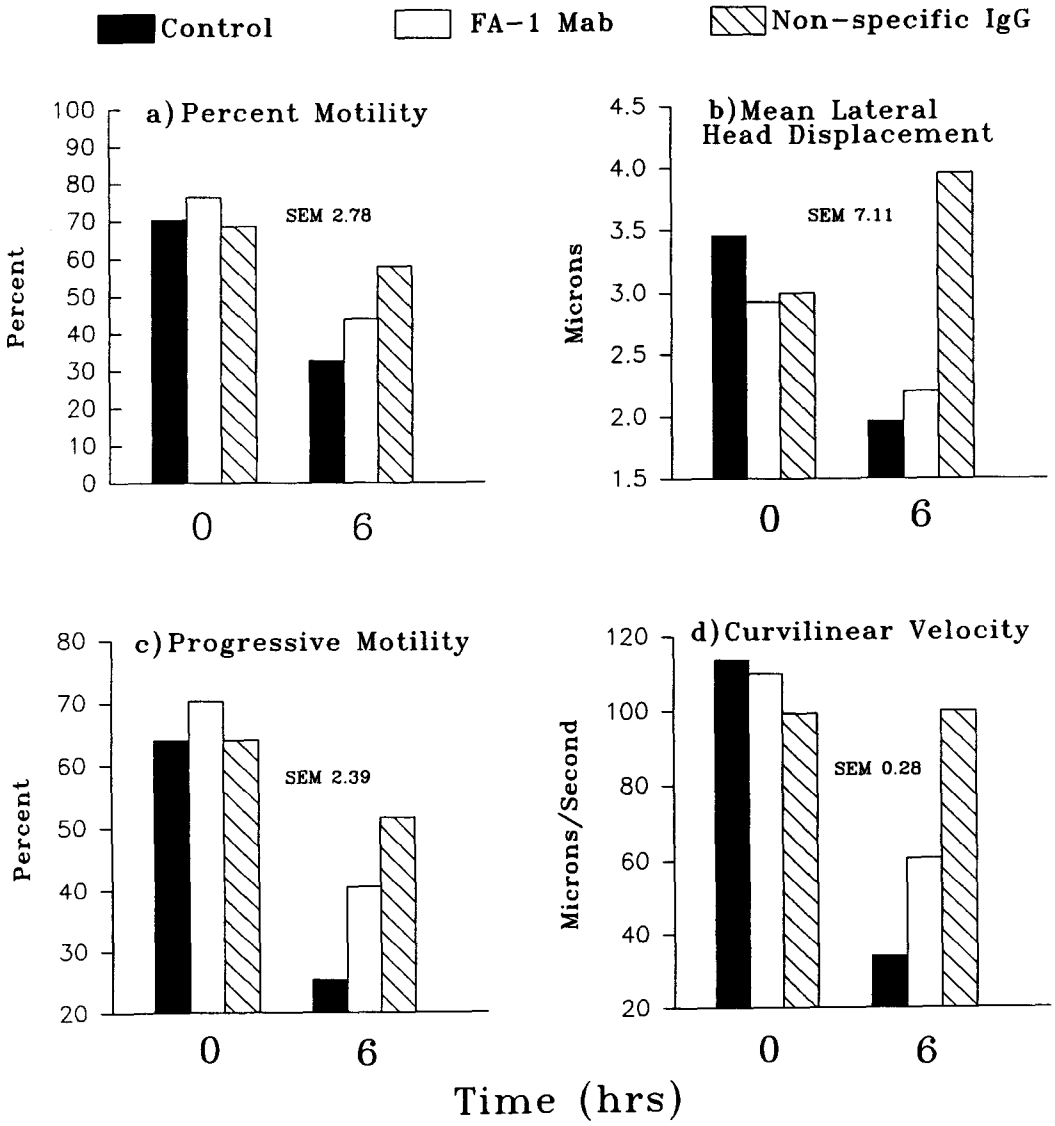

FIG. 5. Effects of nonspecific IgG and FA-1 mAb on a) % motility, b) mean lateral head displacement, c) progressive motility, and d) curvilinear velocity of bovine sperm cells. Sperm were incubated in IVF medium (devoid of oocytes) containing either no IgG, 80 μg/ml of FA-1 mAb, or 80 μg/ml nonspecific IgG for 6 h, and sperm motility parameters were studied by digital image analysis. Values are means from 3 repetitions with each repetition consisting of > 100 sperm. There was no effect of treatment on sperm motion parameters at time = 0 h ($p > 0.10$). Comparison of motility parameters of the treatment groups at t = 6 h showed that the addition of nonspecific IgG ameliorated the reduction in motility parameters apparent in control and FA-1 treatment groups. FA-1-treated sperm were intermediate in response ($p < 0.01$) except for mean lateral head displacement, where control and FA-1 did not differ.

μg of nonspecific IgG/ml of IVF medium and the other containing no antibodies. The three experimental groups had 20 μg/ml, 40 μg/ml, and 80 μg/ml of FA-1 mAb added to the IVF medium. Oocytes were considered "unfertilized" if they had a metaphase II plate (Fig. 4a) or one pronucleus (Fig. 4b). Oocytes that contained two pronuclei (Fig. 4c) or were polyspermic (Fig. 4d) were considered "fertilized."

Fertilization rates for nonspecific IgG (86.7%) and the control containing no antibodies (83.3%) were not different ($p > 0.10$) and were pooled for subsequent analyses. There was a significant ($p < 0.01$) dose-dependent inhibition of fertilization in the three experimental groups (Table 1). The FA-1 mAb groups at 20 μg/ml, 40 μg/ml, and 80 μg/ml mAb concentrations had fertilization rates reduced to 54.6%, 21.6%, and 1.8%, respectively. The data fit the quadratic equation $y = 86.3—4.05X + 0.048 (X)^2$.

Effect of FA-1 mAb on Sperm Motility

Visual assessment of spermatozoa after IVF. There was no visual effect of the FA-1 mAb on sperm motility after 6 h of incubation with oocytes in IVF medium, and the FA-1 mAb did not cause any perceptible agglutination or immobilization of sperm after incubation. When the oocytes were assessed for fertilization rates, it was noted that the FA-1 mAb-treated oocytes had fewer spermatozoa bound to the zona pellucida than did the controls.

Effects of FA-1 and nonspecific IgG mAb on sperm motility parameters. To investigate whether the effect of FA-1 mAb on sperm penetration rates was due an effect on sperm motility parameters, sperm samples containing either 80 μg/ml nonspecific IgG, 80 μg/ml FA-1 mAb, or no antibodies were incubated in IVF medium (devoid of oocytes) as described above, and various motility parameters were studied at t = 0 h and t = 6 h. This experiment was replicated three times, with each treatment group containing > 100 sperm per replication. As expected, there was no effect of nonspecific IgG or FA-1 mAb on % motility (Fig. 5a), mean lateral head displacement (Fig. 5b), progressive motility (Fig. 5c) or curvilinear velocity (Fig. 5d) at t = 0 h ($p > 0.10$). At t = 6 h, all treatment groups showed a decrease in motility parameters ($p < 0.01$) when compared to t = 0 h, except for the nonspecific IgG, which showed no change in mean lateral head displacement or curvilinear velocity ($p > 0.10$). Comparison of the differences in the motility parameters of the treatment groups at t = 6 h showed that the addition of nonspecific IgG ameliorated the reduction in motility parameters apparent in control and FA-1 treatment groups. FA-1-treated sperm were intermediate in response ($p < 0.01$) except for mean lateral head displacement, where control and FA-1 did not differ.

DISCUSSION

Previous reports of the characteristics of FA-1 have shown that in solubilized human testis preparations, FA-1 represents approximately 3.5% of the total protein. FA-1 is a glycoprotein, with carbohydrates representing 18.8% of the total antigen mass. In the testis, where the protein is synthesized, the antigen can be found at various stages of maturation, degradation, or modification (glycosylation) thereby resulting in differently charged species of the same antigenic molecule. Mature human sperm were also shown to contain the same antigen but in relatively low quantities. Further analysis with Western blot procedures showed that the FA-1 mAb reacted with proteins of 23 ± 3 kDa (monomeric form) and/or 51 ± 3 kDa (dimeric form) when detergent-solubilized membrane preparations were used [6, 7].

In this study, Western blot analysis revealed that FA-1 mAb reacted with proteins of 53 ± 2 kDa in all human and bovine samples tested (most likely representing the dimeric form of FA-1). Proteins of 28 ± 3 were detected in the human fresh sperm and bovine testis samples and could possibly represent the monomeric form of the molecule. In the human fresh sperm sample, the antibody reacted with a protein of 79 ± 2 kDa, which may represent the trimeric form of FA-1. In this study, we used whole-sperm preparations for our Western blot analysis rather than swim-up sperm samples as used previously [6, 7]. This difference could explain the appearance of the various forms of FA-1, since the whole-sperm samples would contain spermatozoa at different stages of maturation. Varying degrees of glycosylation may account for the cross-reacting proteins seen in the samples. One difficulty encountered during the gel electrophoresis procedure was that the solubilized sperm preparations contained large amounts of DNA, which interfered with protein migration through the gel. The addition of polyethylenimine to the samples successfully precipitated much of the DNA (which was then removed from the sample) and may have also inadvertently led to the removal of some protein from the sample. This could account for the light banding pattern seen in the human frozen-thawed sample. In all samples tested, the FA-1 mAb reacted with a 53-kDa band; this result concurs with previous publications on the molecular size of FA-1 in humans and other species.

Previous research has shown that FA-1 mAb binds to the post-acrosome, midpiece, and tail regions of methanol-fixed human spermatozoa [6, 7]. In the rabbit, anti-FA-1 antibodies also bind to the same region of the spermatozoa [8]. Results from IFT in this study demonstrated that human FA-1 mAb bound mainly to the post-acrosomal and tail regions of fresh bovine sperm samples. In frozen-thawed bovine samples, it was more difficult to obtain the intensity of fluorescence that was observed in the fresh samples. There was also a higher percentage of spermatozoa that showed only acrosomal fluorescence patterns in frozen-thawed samples (data not shown). The acrosomal fluorescence pattern was also seen in the control nonspecific IgG samples and in samples incubated with secondary antibody alone

(data not shown). This seems to indicate that the acrosomal region may be resistant to washing procedures.

Upon further investigation using confocal scanning microscopy, FA-1 mAb was shown to be bound to the anterior tail regions of the fresh bovine spermatozoa samples as well as to the past-acrosomal region. Laser scanning confocal microscopy not only allowed for greater resolution of FA-l mAb binding sites on spermatozoa but also allowed for a quantitative measurement of the intensity of fluorescence of different regions of the sperm cells.

Previous studies have attempted to ascertain whether antibodies to FA-1 caused a reduction in the fertilizing ability of human spermatozoa by affecting either capacitation, the acrosome reaction, or attachment to and penetration of the zona pellucida. When human sperm were treated with FA-1 mAb, there was a reduction in such sperm motility parameters as amplitude of lateral head movement, velocity, and beat/cross frequency [20]. An increase in these parameters is usually associated with hyperactivation, which is characteristic of capacitation. Results from another report [21] demonstrated that there was a reduction in human sperm-zona binding when the spermatozoa were treated with anti-FA-1 antibodies. These studies, as well as others, indicate that antibodies to FA-1 block fertilization by affecting capacitation and/or the acrosome reaction of sperm cells as well as by reducing sperm-zona binding.

Human FA-1 mAb did not have an inhibitory effect on any of the bovine sperm motility parameters analyzed in this experiment. In this study, visual assessment of fixed oocytes indicated a decrease in the number of sperm cells attached to the zona pellucida in the FA-1 mAb-treated oocytes as compared to the control groups. This suggests that the human FA-1 mAb probably inhibits fertilization in the bovine species by affecting an event after capacitation, possibly sperm-zona binding. In an attempt to better understand the mechanism by which mAb to human FA-1 affect bovine sperm, we are planning experiments in which we will study other motility parameters associated with the onset of capacitation and the acrosome reaction such as the hyperactivation phenomenon. We will also determine the number of sperm undergoing the acrosome reaction in FA-1 mAb-treated sperm as well as quantify the number of FA-1 mAb-treated sperm bound to the zona pellucida.

It is interesting to note that the nonspecific IgG reduced the effect of time on mean lateral head displacement and curvilinear velocity in treated bovine spermatozoa. This result was not totally unexpected, however, since during the IVF trials it was observed that the sperm in the nonspecific IgG samples were highly motile, if not hypermotile, even after 24 h in culture. There may be some as yet unknown nonspecific effect on sperm motility parameters caused by the addition of these nonspecific immunoglobulins and not by specific IgGs such as FA-1.

This is the first report in which oocytes obtained from slaughterhouse ovaries were used to test the effectiveness of mAbs, which were generated against human fertilization-related sperm antigens, in inhibiting fertilization in vitro. The ability to utilize thousands of oocytes weekly for studying the antifertility effects of various mAbs will aid in the process of delineating target antigens to be employed for the development of a suitable contraceptive vaccine for humans and other mammals. This system will also be useful for studying the effects of mAbs on other aspects of fertilization and early embryonic development. Other domestic animals such as dogs [22] and pigs [23] have been used as models for immunocontraception research. Cattle and sheep may also serve as good models for this type of research, due to availability of gametes and tissues as well as access to large numbers of animals for in vivo trials.

In conclusion, our data further demonstrate that FA-1 is an evolutionarily conserved antigen that plays an important role in the fertilization process, as demonstrated by the dose-dependent reduction in fertilization rates in bovine IVF trials with an almost complete inhibition of fertilization at the highest mAb concentration. The bovine FA-1 antigen was also shown to be present in the same region and to have a molecular size similar to that of human FA-1. These results indicate that FA-1 is a promising candidate for the development of a contraceptive vaccine. The research also suggests that the bovine species could possibly be used as a model for investigating the use of FA-1 as an immunovaccine in nondomestic animals in which there are significant overpopulation problems. We are currently utilizing polyclonal antibodies generated against human FA-1 to purify bovine FA-1 for use in in vivo antifertility trials in the bovine and ovine species and possibly other ungulates such as deer.

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