Evidence for a Role of Post-Ovulatory Cumulus Components in Supporting Fertilizing Ability of Hamster Spermatozoa

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A completely chemically defined culture medium was used to investigate the role of egg-cumulus complex (ECC) components in supporting sperm fertilizing ability. Defined sperm motility-stimulating factors (hypotaurine and epinephrine), with polyvinylalcohol as the macromolecular component, were included in the defined medium. Freshly-ovulated hamster ECCs were incubated with washed epididymal spermatozoa under different conditions designed to evaluate the ability of ECC components to support sperm capacitation, acrosome reactions, and the ability to penetrate the ova. The major conclusions from the data are that ECC components are capable of supporting these physiologic events, and that these components are present in the soluble (fluid) compartment of the ECC. This work is the first in a series of steps aimed at the localization, characterization, and eventual identification of the natural acrosome reaction-inducing stimulus associated with the ECC.

Key words: fertilization *in vitro*, capacitation, acrosome reaction, egg-cumulus complex, golden hamster.

The acrosome reaction of the fertilizing mammalian spermatozoon normally takes place within the oviduct during penetration of the egg investments. Whether this crucial event occurs while the spermatozoon is passing through the cumulus oophorus or following the binding of the sperm to the zona pellucida is presently controversial, since good evidence exists to support both possibilities (Bedford, 1968, 1970; Saling et al, 1978; Yanagimachi, 1981; Cummins and Yanagimachi, 1982). Resolution of this question is critical to our understanding of the functional significance of the acrosome reaction in mammals, of the role of the acrosomal enzymes, and of the location and nature of acrosome reaction-inducing substances within the egg-cumulus complex (ECC). The ECC comprises the cumulus oophorus and corona radiata, the matrix material connecting these cells, the fluid contained in the interstices of the matrix, and the ova enclosed in the zonae pellucidae. The existence of acrosome reaction-stimulating substances in the ECC is implied by the close relationship between the occurrence of the reaction and sperm penetration of the egg investments. Most oviductal spermatozoa that are temporally and/or spatially separated from the freshly-ovulated ECC do not exhibit acrosome reactions, whereas all spermatozoa that have penetrated the zona pellucida have undergone acrosome reactions (Austin and Bishop, 1958; Yanagimachi, 1981). If the hypothesis that specific ECC components induce the acrosome reaction is correct, it is logical to infer that these substances are not present (or exist in a suboptimal condition) in the oviductal secretions; otherwise, the synchronization of acrosome reactions and sperm penetration of the ECC would be impaired or lost.

Experiments designed to demonstrate acrosome reaction-inducing properties of the ECC have not been conclusive—for a variety of technical reasons.

This paper is dedicated with gratitude and love to the memory of Pierre Soupart, who was a treasured friend, colleague and mentor.

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Localization of acrosome reaction-inducing substances within the ECC, hopefully followed by their isolation and identification, could be invaluable to our understanding of the mechanism of the acrosome reaction, which is presently very limited, and might also throw light on the functional significance of this reaction. The present work was undertaken as the first of a series of steps towards these goals, and was intended to demonstrate whether or not functional acrosome reaction-inducing substances are associated with the post-ovulatory ECC. The hamster was used because of the high degree of reproducibility of *in vitro* fertilization procedures with this species (Leibfried and Bavister, 1981). The design of this study makes use of the recent demonstration that the synthetic polymer polyvinylalcohol (PVA) can be used to eliminate the need for protein (bovine serum albumin, BSA) in the culture medium to maintain sperm motility (Bavister, 1981a, 1981b). This substitution completely avoids the complications arising from the potent acrosome reaction-inducing properties of BSA (Meizel, 1978). Furthermore, chemically defined factors (hypotaurine and epinephrine) were used to maintain sperm motility (Meizel et al, 1980), thus eliminating dependence on ECC components for this function, which was a major complication in an earlier study (Yanagimachi, 1969a).

This defined culture system allows the acrosome reaction-inducing properties of the hamster ECC to be studied *in vitro*, independently from its sperm motility-sustaining role. The present study is the first to report separation of these functions using defined *in vitro* conditions.

Materials and Methods

Culture Medium

The culture medium used in these experiments was specially developed for hamster *in vitro* fertilization. This medium, designated TALP, is a modified Tyrode's solution with increased sodium bicarbonate (total 25 mM), containing 10 mM sodium lactate and 0.1 mM sodium pyruvate (Bavister and Yanagimachi, 1977). For the present study, PVA (1 mg/ml) was used, instead of BSA (Bavister, 1981b), to provide a completely chemically defined culture medium (medium TLP-PVA). BSA (fraction V fatty acid-free) (Miles-Pentex Laboratories, Elkhart, Indiana) was sometimes added as a supplement to this medium (medium TALP-PVA). PVA (cold water soluble, Type II) (Sigma Chemical Co., St. Louis, Missouri) was treated by passing a 5% solution in water through a 10 ml column of Chelex-80 (Biorad, Richmond, California) to remove any trace-metal impurities. The filtered PVA solution was lyophilized, then made up as a stock solution (100 mg/ml) in 150 mM NaCl solution. Aliquots of this solution were stored at −20 C.

Preparation of Sperm Suspensions

Spermatozoa were collected by puncturing the distal tubules (under mineral oil) of excised caudae epididymides that were taken from 3- to 6-month-old golden hamsters. A 10 μl aliquot of the cauda epididymal contents was suspended in 10 ml of "sperm washing medium" (SWM) in a 15 ml plastic centrifuge tube. SWM consists of equal volumes of Dulbecco's phosphate-buffered saline (*p*H 7.2) and 290 mM sucrose solution (Bavister and Yanagimachi, 1977), to which 1 mg/ml PVA was added. The spermatozoa were gently dispersed into the SWM by rocking the centrifuging tube then centrifuged at 200 × g for 5 minutes. The supernatant was discarded and the sperm pellet was mixed with an equal volume of fresh SWM (final volume approximately 0.5 ml). The dilution of cauda epididymal plasma, resulting from this sperm washing procedure, was approximately 1/4000. Each suspension that was prepared this way, using sperm from one male hamster, was used to inseminate all treatment drops in each replicate, which consisted of two duplicate observations of each treatment. A total of three male hamsters was thus used to provide sperm for all observations reported in this study.

Recovery and Treatment of ECCs and Ova

ECCs were recovered under mineral oil from superovulated hamsters (Leibfried and Bavister, 1981) 16 hours after HCG injection. ECCs from two or three female hamsters were pooled into a single large mass, which was subdivided using fine watchmaker's forceps into six portions of approximately equal size (1−2 μl). Two of these ECC portions were transferred into separate 45 μl drops of TLP-PVA, that had been equilibrated with 5% CO_2 in air, and were contained in a 60 × 15 mm plastic Petri dish (Falcon Plastics, Oxnard, California) under mineral oil. These drops, which were supplemented just before insemination, formed the basis for ECC treatments 2 and 3 (Table 1). Two more ECC portions were transferred similarly to drops of TALP-PVA (treatments 5 and 6, Table 1). Another ECC portion was transferred to 3 ml of equilibrated TLP-PVA, overlaid with 2 ml of mineral oil, and was incubated under 5% CO_2 in air for 5−10 minutes, with occasional vigorous agitation using a siliconized Pasteur pipet; it was then transferred to a second dish in which the process was repeated. Following this rinsing procedure, the ECC portion was transferred to a 45 μl drop of TLP-PVA (treatment drop 4, Table 1) contained in the same Petri dish as the other treatments. The sixth and final ECC portion was transferred to 3 ml of TLP-PVA, containing 3 mg hyaluronidase; after 5−10 minutes, the naked ova were rinsed three times in separate 3 ml volumes of TLP-PVA. Finally, the ova were placed in a 45 μl drop of TLP-PVA (treatment drop 1, Table 1) in the same dish as all the other treatments. Thus, all six treat-

TABLE 1. Influence of Post-Ovulatory ECC on Fertilization of Hamster Ova *In Vitro*

Treatment Number	Conditions of Incubation: Macromolecule in Culture Medium	PHE*	Cumulus†	Total Number of Ova Inseminated	Total Number of Ova Penetrated (mean % = SEM)‡§	Total Number of Ova Fertilized (mean % ± SEM)§‖
1	PVA	+	−	46	0 (0)	0 (0)
2	PVA	−	+	56	11 (17.3 ± 8.5)**	0 (0)
3	PVA	+	+	76	61 (79.3 ± 6.2)††‡‡	16 (21.5 ± 9.3)**
4	PVA	+	+¶	79	17 (22.2 ± 8.8)**	0 (0)
5	PVA + BSA	+	+	76	74 (97.2 ± 1.8)‡‡	62 (80.7 ± 5.4)††
6	PVA + BSA	−	+	70	43 (56.3 ± 14.4)††	23 (34.2 ± 12.1)**

* Presence (+) or absence (−) of defined sperm motility factors (penicillamine, hypotaurine, and epinephrine).
† Presence or absence of cumulus oophorus and corona radiata and their matrix components.
‡ All stages of penetration included (sperm in perivitelline space and/or sperm in vitellus).
§ Values given are percentages derived from three replicate experiments, each with two duplicates (total of six observations for each treatment).
‖ Ova with sperm in vitellus, two well-developed pronuclei and two polar bodies. Sub-class of "ova penetrated" category.
¶ ECC thoroughly rinsed before incubation.
**·††·‡‡ Values with different superscript symbols within each column are significantly different from each other ($P < 0.05$).

ment drops were contained in the same dish and were inseminated at same time from the same sperm suspension.

Insemination of Drops

When all six treatment drops had received ECCs or ova, 2 μl of sterile 0.9% sodium chloride solution (treatment drops 2 and 6, Table 1) or 2 μl of a chemically defined sperm motility factor preparation (treatments 3, 4 and 5) or 2 μl of each (treatment 1) were added. The motility factor preparation consisted of D-penicillamine, hypotaurine, and epinephrine (500 μM, 250 μM, and 25 μM, respectively) stabilized with sodium lactate and sodium metabisulfite (Leibfried and Bavister, 1982). This stock mixture is designated PHE. The components of PHE were obtained from Sigma Chemical Co. (St. Louis, Missouri). Immediately after addition of saline or PHE, all treatment drops in a single dish were inseminated with 1−2 μl of the washed sperm suspension, giving a final sperm concentration of $2-4 \times 10^5$/ml. The dish was then incubated at 37 C under 5% CO_2 in air for six hours. Ova were then fixed by addition of 50 μl of neutral-buffered formalin to each of the treatment drops, thus stopping the processes of gamete interaction in all drops at the same time. Dishes were stored at 4 C overnight, and were then examined for evidence of sperm penetration and fertilization (Bavister, 1980), using phase-contrast and differential interference contrast microscopy.

The time-interval between recovery of ECCs from the oviducts to insemination of treatment drops was 30−60 minutes. All treatment drops contained in a single Petri dish were inseminated within 30 seconds of each other.

The sperm concentrations used in this study with intact ECCs were approximately 10-fold higher than those used in previous experiments with naked oocytes (Leibfried and Bavister, 1981, 1982) because a high proportion of the spermatozoa became associated with the cumulus cells, apparently irreversibly, and thus were not available for penetration of the ova. Attempts to use lower sperm concentrations than those reported here with intact ECCs have resulted in few or no ova being penetrated.

Experimental Design and Analysis of Data

The study utilized six treatments. A total of six drops of culture medium, each corresponding to one treatment, was contained in a single Petri dish. Before assignment to treatment drops, ECCs from different female hamsters were pooled to reduce between-oviduct and between-female variability in the fertilizability of ova and other properties of the ECCs. Following insemination of all the treatment drops in a dish, a second dish identical to the first was prepared, using ECCs from two or three more female hamsters. The two dishes constituted one replicate of the experiment. Three replicates were performed for a total of six observations for each of the six treatments. Proportional data (percentages of ova penetrated or fertilized) were transformed into angles by the arc-sin transformation. Analysis of variance was performed for one-way classification using five or three treatment groups (Table 1, ova penetrated and ova fertilized, respectively; treatments yielding all zero responses were eliminated from the analysis). Significant differences between treatments were tested by Duncan's multiple range test. Both analyses were performed using the SAS computing program (Barr et al., 1976).

Results

All of the ova penetration data obtained in this study are presented in Table 1. The motility of spermatozoa was well maintained in all treatment drops containing PHE (sperm motility factor preparation). The efficacy of this preparation was evidenced by the observation that an estimated 60–80% of the spermatozoa that were incubated in treatment 1 were still vigorously motile at the end of the six hour incubation period. In this treatment, the ova were densely covered with attached spermatozoa within minutes after insemination. However, none of the ova inseminated under these conditions was penetrated (Table 1). Virtually every one of the spermatozoa attached to the naked ova in treatment 1 had an intact acrosome, as observed by phase-contrast microscopy, and most of these spermatozoa were easily dislodged from the zonae pellucidae by drawing the ova in and out of a fine-diameter Pasteur pipet.

Spermatozoa incubated under the conditions of treatment 2 (no PHE, intact ECC) exhibited poor motility compared to those in treatment 1 (Table 1). A low percentage of ova were penetrated (17.3%), but none showed advanced stages of fertilization (pronuclei). In contrast to the results obtained in treatments 1 and 2, spermatozoa that were incubated with PHE and intact ECC (treatment 3) penetrated a high percentage of ova, and 21.5% of ova were at the pronuclear stage. A strikingly different result was obtained when washed ECC were used (treatment 4). Although sperm motility was well sustained and sperm attached in considerable numbers to the zonae pellucidae, only 22% of ova were penetrated, and none reached the pronuclear stage within six hours.

Comparative data were provided by treatments 5 and 6. Nearly all ova were penetrated and most were at the pronuclear stage by six hours, when BSA was included in the culture medium (treatment 5). By comparison, the percentages of penetrated and of fertilized ova were significantly reduced when PHE was absent (treatment 6).

In treatments (3, 4 and 5) containing PHE, the cumulus oophorus and corona radiata were rapidly dispersed (15 to 30 minutes) by vigorously motile spermatozoa, and ova were observed to have numerous (approximately 10–30) spermatozoa attached. Due to the pronounced affinity of spermatozoa for the dispersed cumulus cells, the numbers of freely motile sperm available for interaction with ova were much lower than the total number of motile sperm introduced into each treatment drop (treatments 2–6). By approximately five hours after insemination of treatment drops containing cumulus/corona cells, nearly all spermatozoa were attached either to these cells or to the zonae pellucidae of ova. It was not possible to make precise counts of the numbers of acrosome-reacted spermatozoa that were attached to ova. Examination of sample ova, incubated in treatments 3 and 5 (PHE together with intact ECC) between five and six hours after the beginning of incubation, showed that many of the attached motile sperm (which could not be easily dislodged by pipetting) had undergone acrosome reactions. This was in striking contrast to the situation in treatment 1, in which virtually no acrosome-reacted spermatozoa were found on the ova, even though the ova were densely covered with sperm.

Discussion

It is believed that the role of the acrosomal enzymes is to assist the fertilizing spermatozoon in its passage through the egg investments. It follows that the release of these enzymes during the acrosome reaction needs to be synchronized with the presence of the ECC in the immediate vicinity of the sperm. The obvious mechanism for accomplishing this objective is for some component of the ECC itself to initiate the acrosome reaction in suitably prepared (capacitated) spermatozoa. Morphologic evidence, consistent with this notion, has been provided by studies of rabbit sperm/ECC interaction *in vivo*. Most spermatozoa within the oviductal ampulla that were distant from the ECC had intact acrosomes, whereas the majority of spermatozoa passing through the cumulus oophorus had undergone at least partial acrosome reactions (Bedford, 1968, 1970). A somewhat different picture is apparent from a recent study using the golden hamster, in which motile spermatozoa, showing acrosomal modification, were found both in the fluid within the oviductal ampulla and in the cumulus itself (Cummins and Yanagimachi, 1982). Therefore, this study did not point unequivocally to the ECC either as the site of the acrosome reaction or as the principal source of an acrosome reaction-initiating factor.

A possible role of ECC components in stimulating acrosome reactions in fertilizing sperma-

tozoa may be more readily demonstrated *in vitro*. Attempts to achieve this objective, however, have not been entirely conclusive. Preovulatory ECCs, recovered from hamsters, were able to induce acrosome reactions and to support fertilization *in vitro;* and factor(s) that were responsible appeared to be contained in the follicular fluid component of the ECC (Barros and Austin, 1967). Although this and later work (Yanagimachi, 1969a, 1969b) attributed follicular fluid with acrosome reaction-stimulating properties, it is debatable whether or not follicular fluid—as such—is present as a component of the post-ovulatory ECC; thus its role in supporting sperm penetration under natural conditions is questionable. One of the most detailed analyses of individual (preovulatory) ECC components pointed to the fluid component as the sole source of factor(s) that supported fertilizing ability of hamster spermatozoa (Yanagimachi, 1969a). By contrast, another study using hamster gametes indicated that the cumulus cell component of hamster (postovulatory) ECC was the site of the active substance(s) responsible for supporting fertilizing ability of spermatozoa (Gwatkin et al, 1972).

An inherent problem in experiments that use ECCs to support sperm fertilizing ability complicates the interpretation of these and other results, and probably underlies the conflicts apparent in data from different studies, as described above. This problem derives from dual properties of the hamster ECC, which not only may possess acrosome reaction-stimulating components, but may also be involved in supporting viability and vigorous motility of spermatozoa. The latter property of ECC becomes apparent only when the culture medium lacks protein and sperm motility-stimulating factors, as in all of the studies cited above. Thus, in one study using washed ECCs, there was a pronounced decrease in sperm motility, concomitant with the loss of fertilizing ability (Yanagimachi, 1969a). In another investigation, separate sperm motility-stimulating and acrosome reaction-inducing properties were described in heterologous (bovine) follicular fluid (Yanagimachi, 1969b). A major question that remained unanswered by all of these studies is whether, in the absence of particular components of hamster ECCs, an observed failure of sperm-fertilizing ability is due simply to a loss of sperm viability or to the absence of ECC components that stimulate acrosome reactions, or to both. The answer to this question is critical to the attribution of a functional role of post-ovulatory ECC components in stimulating acrosome reactions in fertilizing spermatozoa.

The present study was designed to provide unequivocal information on this subject. The sperm motility-stimulating properties of the hamster ECC, which are essential for fertilization to occur *in vitro* (Yanagimachi, 1969a), were replaced by the chemically defined sperm motility factors hypotaurine and epinephrine (Meizel et al, 1980). In addition to these factors, hamster spermatozoa require macromolecules for their survival *in vitro*. This requirement can be satisfied without relying on ECC components by incorporating BSA in the culture medium, but BSA in addition to supporting sperm viability also stimulates acrosome reactions very effectively, which would be self-defeating in the present context. This difficulty was avoided by incorporating PVA in the culture medium (Bavister, 1981a, 1981b). Thus, the culture system that was used in the present study consisted of a balanced salt solution based on Tyrode's solution, with the addition of PVA, hypotaurine, and epinephrine. When all of these chemical substances were present, vigorous sperm motility was always maintained, regardless of the presence or absence of ECC components. Washed sperm were used to eliminate contributions from cauda epididymal plasma, that could influence sperm motility and/or acrosome reactions (Bavister et al, 1978). Under these conditions, dispersion of cumulus cells by spermatozoa allowed direct interaction of the sperm with the zonae pellucidae of ova. This situation is not comparable with that pertaining *in vivo* in the hamster, in which the fertilizing spermatozoa must penetrate through the intact cumulus before reaching the zonae pellucidae of ova. A modification of these procedures, in which minute numbers of sperm are added to intact ECCs in a microchamber, is being developed. This modification will allow observations to be made on the progression of fertilizing spermatozoa through the cumulus.

In the chemically defined culture medium (TLP-PVA), ova were not penetrated by spermatozoa in the absence of other ECC components (Table 1, treatment 1). This observation confirmed the results obtained in a previous study (Bavister, 1981b). Virtually all spermatozoa attached to the ova retained intact acrosomes throughout the ga-

mete incubation period. When intact ECCs were present, 79% of the ova were penetrated within six hours (treatment 3), and many of the spermatozoa that were attached to the zonae pellucidae had undergone acrosome reactions. These observations clearly implicate ECC components in stimulating acrosome reactions of hamster spermatozoa, as well as in supporting capacitation of spermatozoa prior to occurrence of acrosome reactions. The efficiency of ECC components in supporting sperm fertilizing ability was considerably less than that found when BSA was present in addition to ECC (treatment 5). Although the percentages of penetrated ova were not significantly different, the proportion of ova with pronuclei was much greater in the presence of BSA (80.7% vs. 21.5% without BSA). This discrepancy may be accounted for by dilution of ECC components (approximately 1/25−1/50) in the culture medium. A reduction in the incidence of fertilization was also noted by Yanagimachi (1969a) with progressive dilution of ECCs.

The importance of including sperm motility factors in the culture medium can be seen by comparing results obtained with treatments 2 and 3. In the absence of PHE, the efficiency of intact ECCs in supporting sperm fertilizing ability was low, and no pronuclear ova were found (treatment 2). This efficiency was increased more than 4-fold in the presence of PHE, and 21.5% of ova were pronucleate (treatment 3). This difference clearly illustrates the need to provide a means of maintaining sperm motility when evaluating the acrosome reaction-stimulating properties of the ECC. Even when BSA was also present in the culture medium, which considerably increased the proportions of penetrated and of fertilized ova (compare treatments 6 and 2), there were significant additional effects in both categories of adding PHE (treatments 5 vs. 6).

The ECC factors supporting sperm fertilizing ability (capacitation and acrosome reactions) are apparently located in the soluble component of the ECC. When ECCs were thoroughly washed before insemination, only 22.2% of ova were penetrated, and none reached the pronuclear stage of fertilization within six hours (treatment 4), although vigorous sperm motility was maintained. Quantitation of acrosome reactions in spermatozoa attached to these ova was not possible, but it was judged that the proportion of acrosome-reacted spermatozoa was lower in this situation than in treatments containing intact ECCs and/or BSA. In preliminary experiments (Bavister, unpublished data), hyaluronidase-treated washed cellular components of the ECC (cumulus and corona cells) were not able to support the fertilizing ability of hamster spermatozoa that were incubated in TLP-PVA with PHE.

These observations demonstrate the existence of substances within the fluid component of the post-ovulatory hamster ECC that can support capacitation and stimulate acrosome reactions in hamster spermatozoa. Experiments are now in progress to determine if washed cumulus/corona cells can regenerate these substances, and to provide information on their physico-chemical properties. This approach, using ovulated ECCs, is more difficult to perform technically than the alternative of using BSA to stimulate acrosome reactions (Meizel, 1978). However, it provided a means to obtain information on the naturally occurring acrosome reaction-stimulating substances that are associated with the ECC, whereas the mode of acrosome reaction stimulation by BSA or by other exogenous molecules may not necessarily be identical.

Although data obtained from *in vitro* studies cannot be directly extrapolated to the *in vivo* situation for obvious reasons, these results may nonetheless be relevant. In a study in which hamster ova were transferred to the oviducts of mated hamsters, a significant decrease was noted in the percentage fertilization when naked ova, compared to cumulus-intact ova, were used (Moore and Bedford, 1978). This difference was virtually or completely eliminated when follicular fluid or blood serum accompanied the naked ova that were transferred to the oviducts. One interpretation of these data is that some component of the ECC is important (although not obligatory) for supporting sperm fertilizing ability. Apparently, soluble factor(s) that are found in follicular fluid and blood serum can replace the ECC component. It may be that, *in vivo*, ECC components help to provide optimal conditions for the accomplishment of fertilization. It is not clear if constituents of follicular fluid are present as components of post-ovulatory ECCs and if they could thus be responsible for stimulating acrosome reactions in the fertilizing spermatozoa. Although follicular fluid comprises the fluid component of the ECC at the time of ovulation, it is neither known how rapidly this fluid may be replaced by oviductal fluid compo-

nents, nor to what extent follicular fluid constituents (for example, proteins) remain trapped within the cumulus. Certainly, in the present study, the fluid component(s) of the ECC that were capable of stimulating acrosome reactions and supporting sperm fertilizing ability were readily removed by rinsing ECCs in culture medium. If elution of follicular fluid from the ECC by oviduct fluid takes place within the oviduct, it is possible that follicular fluid components of the ECC play a role in supporting fertilization, which is gradually taken over by oviduct fluid components that have the same function. Such a mechanism could represent a "back-up" system, ensuring the success of fertilization in the event that spermatozoa are delayed in reaching the ampulla. At the present time, however, the absence of critical information on the composition of the fluid component of the ECC precludes ascribing a physiologically significant role in fertilization to follicular fluid constituents, in spite of the pronounced ability of this fluid to stimulate acrosome reactions (Yanagimachi, 1969a, 1969b; Meizel, 1978).

Under the conditions used in this study, hamster spermatozoa in treatment 1 attached to the zonae pellucidae of naked ova in the absence of all other ECC components and of BSA. However, these spermatozoa were readily dislodged, did not exhibit acrosome reactions, and were not able to penetrate the zonae. These observations indicate that the hamster zona pellucida is not an effective inducer of acrosome reactions in attached spermatozoa, and they militate against the notion that the zona pellucida is the site of the acrosome reaction during sperm penetration of the intact ECC. A completely different result is obtained when BSA is also present in the culture medium under otherwise identical conditions to those used here—sperm attach firmly to the zonae pellucidae of naked ova, undergo acrosome reactions, and accomplish fertilization (Bavister, 1981b). These data indicate a need for very careful interpretation of experiments, using BSA-containing culture media, that are aimed at delineating the site of acrosome reactions during sperm penetration of egg investments, and of experiments aimed at localizing acrosome reaction-stimulating components of the ECC. This caveat may also apply to experiments with mouse gametes, since in this species, few sperm are able to undergo acrosome reactions, and no penetration of cumulus-free ova occurs unless BSA is present in the culture medium (Miyamoto and Chang, 1973; Hoppe and Whitten, 1974; Saling et al, 1978).

In the present study, a new approach has been used to evaluate the properties of post-ovulatory ECCs. This approach employs a completely chemically defined culture medium containing sperm motility factors (epinephrine and hypotaurine) and PVA in order to eliminate dependence on ECC factors for maintenance of sperm viability and motility. In this way, acrosome reaction-inducing properties of the ECC can be studied independently from motility-stimulating properties. It is hoped that these preliminary results will provide a basis for critical reevaluation of important questions concerning the site and mechanism of the acrosome reaction in fertilizing mammalian spermatozoa.

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Pan American Conference on Fertility and Sterility

The Pan American Conference on Fertility and Sterility will be held February 1–9, 1983, at the Acapulco Princess Hotel and Club de Golf, Acapulco, Mexico. A postgraduate course will be held February 1. Accreditation is ACOG 35 cognates plus 6 postgraduate credits. AAFP accreditation has been applied for.

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