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Immunofluorescent Localization of Immunoglobulins on the Cell Surface of Mouse Oocytes and Preimplantation Embryos

LYNN M. WILEY[1,2] and MICHAEL FEMI OBASAJU[1]

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Living mouse oocytes and preimplantation embryos were assayed by indirect immunofluorescence for their ability to adsorb heterologous serum proteins from culture media to their cell surfaces. Bovine and human immunoglobulins of the IgG class were adsorbed by the oocytes and all stages of preimplantation embryos, while IgG of mouse or goat origin was not. In contrast, none of the serum albumins was adsorbed to the cell surfaces of mouse oocytes and preimplantation embryos. From the differential binding of IgG of some, but not all, of the species that were tested, we concluded that these cell surface IgG "receptors" on mouse oocytes and preimplantation embryos are likely to be heterophilic in nature. Similar observations were made irrespective of the strain of mice used to provide the oocytes and embryos. These observations raise the question of whether human oocytes and preimplantation embryos should also be assayed for their ability to adsorb animal serum proteins that are possible candidates as a substitute protein supplement for human serum in culture medium used in human in vitro fertilization/embryo transfer programs.

KEY WORDS: IVF culture media; protein supplement; cell surface immunoglobulin adsorption; mouse embryo culture; heterophil antigens.

INTRODUCTION

Most in vitro fertilization and embryo transfer (IVF-ET) programs supplement their culture media with protein, most often in the form of whole serum. However, there have been several reports indicating that serum confers no advantage to the development of mammalian preimplantation embryos in vitro (1,2) and can be embryo toxic (3). Consequently, our laboratory, as well as several others, has been testing purified serum fractions or proteins in defined culture media for their ability to support the development of mouse preimplantation embryos (1–3), which several programs use for screening media components intended for human embryo culture (4,5).

Conceptually, there are several criteria that should be met by animal serum proteins that are possible candidates for protein supplements in media used to culture human embryos. These criteria include (i) the ability to support maturation and fertilization of human oocytes and preimplantation development of the fertilized human ovum, (ii) lack of toxicity to the mother or to the personnel working with the proteins, (iii) adequate availability, and (iv) ease of preparation. In addition, these protein supplements should not be adsorbed by the oocyte or embryo because of the potential for provoking an immune response should the adsorbed protein be introduced into the uterus during embryo transfer.

This report presents data on the localization by indirect immunofluoresence of heterologous serum albumins and immunoglobulins on the surface of mouse oocytes and preimplantation embryos. As a starting point, we selected albumins and immunoglobulins because our previous work showed that a combination of these serum proteins significantly enhances the developmental rate and embryo cell number of cultured mouse preimplantation embryos (6). In addition, we used several different

[1] Division of Reproductive Biology and Medicine, Department of Obstetrics and Gynecology, University of California, Davis, California.
[2] To whom correspondence should be addressed at the Division of Reproductive Biology and Medicine, Department of Obstetrics and Gynecology, University of California, Davis, California 95616.

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strains of mice differing in H-2 haplotype, which can also influence the rate of embryo development (7,8), to determine whether, in principle, the genotype of an embryo might affect the cell surface adsorption of serum albumins and/or immunoglobulins.

MATERIALS AND METHODS

Mice

C57BL/6j, BALB/cbyj, and CBA/HT6 mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Random bred mice were of the Swiss ICR (CD-1) type and were purchased from Charles River Laboratories, Inc., Shaver Road, Michigan.

Collection of Oocytes and Embryos

Female mice of all strains were superovulated with 5 IU pregnant mare serum (PMS) followed 48 hr later by 5 IU human chorionic gonadotropin (hCG). After hCG injection, each female was placed with an individual male of the same strain and checked for the presence of a vaginal plug the next morning. The plug-positive females were sacrificed 27 to 30 hr later by cervical dislocation. Embryos at the two- to four-cell stage (50 to 54 hr after hCG) were flushed from dissected oviducts with modified Hanks balanced salt solution (BSS) (9) containing 0.5% polyvinylpyrrolidone (PVP). Morulae stage embryos were obtained either from cultured two- to four-cell-stage embryos or were flushed from the dissected uterine tubes of superovulated mice 80 to 82 hr after hCG. Blastocysts were obtained either from cultured two- to four-cell-stage embryos (120 hr after hCG) or from cultured morulae after 20 hr or were flushed from dissected uterine tubes of superovulated mice (92 to 94 hr after hCG). Unfertilized oocytes were gently teased out from the oviducts of unmated superovulated mice 22 to 44 hr after hCG. The oocyte mass was transferred into BSS containing 0.5% hyaluronidase and the oocytes were washed three times in BSS containing 0.5% PVP.

Culture Media

The base culture medium used was the T6 ovum culture medium of Quinn *et al.* (10), which was modified to accommodate the different proteins tested (Table I). All proteins except where noted were obtained from Miles Laboratories, Inc., Elkhart, IN. The other culture reagents were obtained from Sigma Chemical Company, St. Louis, MO. Water was supplied from a Milli-Q RO4 reagent water system equipped with ion exchangers and charcoal filters. All media were used within 30 hr of preparation.

Indirect Immunofluorescence

The primary antibody in all cases was raised in rabbits and, unless otherwise noted, affinity purified against its respective immunogen by the

Table I. Immunofluorescent Localization of Foreign Serum Proteins on the Cell Surface of Mouse Preimplantation Embryos and Unfertilized Eggs: Outbred Mice

			Results[a]			
			Embryo			Unfertilized eggs (180)
Protein in culture	Primary antibody	Secondary antibody	2–4 cell (360)[b]	Morula (360)	Blastocyst (360)	
BSA	α-BSA	R-GAR[c]	−	−	−	−
BSIG	α-BSIgG	R-GAR	+	+ +	+ +	+
Goat IgG	α-Goat Ig	R-GAR	−	−	−	−
Human IgG	α-HSIgG	R-GAR	+	+ +	+ +	+
Mouse serum[d]	α-Mouse Ig (goat)	R-RAG[e]	−	−	−	−
Fetal calf serum (FCS)	α-BSA	R-GAR	±	±	±	±
FCS	α-BSIgG	R-GAR	+	+ +	+ +	+

[a] +, positive fluorescence; −, negative fluorescence; ±, slight fluorescence attributed to background staining.
[b] Total numbers of oocytes/embryos assayed per "protein in culture."
[c] GAR, goat anti-rabbit immunoglobulins (IgG fraction).
[d] Serum contamination introduced during isolation of oocytes and embryos from dissected oviducts.
[e] RAG, rabbit anti-goat immunoglobulins (IgG fraction).

vendor and used at a dilution of 1:4 in BSS. The secondary antibody consisted of the Fab fragment of affinity-purified IgG (affinity chromatography performed by the vendor) and was raised in goats, conjugated with rhodamine, and used at a dilution of 1:100 in BSS. Both antibodies were obtained from Cappel Laboratories, Westchester, PA, unless otherwise noted.

Oocytes and embryos (all stages) were cultured in T6 modified with different proteins (Table I) in plastic 60-mm culture dishes (Falcon Plastics, Cat. No. 3027) at 36.5°C under humidified 5% CO_2, 95% air for 2 hr. After incubation they were washed three times in BSS and then incubated with their respective primary antibody for 30 min in microdrops in plastic dishes at room temperature. At the end of this incubation, the embryos and oocytes were washed three times in BSS and then treated for 15 min with rhodamine-conjugated secondary antibody.

Various controls were run simultaneously in which either the protein in the culture medium or the primary and/or the secondary antibody was changed (Table II). To check for the specificity of labeling by the primary antibodies, they were exhaustively absorbed by incubating them with their respective immunogen proteins at room temperature, then centrifuging at 13,000*g* for 15 min and the supernatant was tested for residual labeling of embryos.

Putative labeled embryos were washed three times in BSS and transferred to glass coverslips containing drops of BSS. After all immunolabeling steps had been completed, the zonae pellucidae were removed mechanically by mouth pipette, except for unfertilized oocytes, where the zonae were removed with pronase (11). Control experiments indicated that pronase did not influence the adsorption of serum proteins by oocytes.

Labeled embryos were then viewed through a Nikon phase-contrast microscope equipped with epifluorescence and filters for rhodamine fluorescence. Photographs were taken with Kodak Tri-X 135-mm film using 1-min exposures and developed in Kodak Microdol-X developer.

RESULTS

General Comments

Methods of general application regarding the timing of dissections and handling of reagents and embryos have been highlighted elsewhere (6) and were followed in this investigation. An average of 20 oocytes or embryos was used for each of the experimental and control groups. The zonae pellucidae in all cases were very brightly labeled, hence their removal for proper visualization of the surface of the oocytes and embryos. The binding of different serum proteins to mouse oocytes and embryos is presented in Tables I–III and represents the summary of three different experimental periods.

Table II. Control Assay for the Immunofluorescent Localization of Foreign Proteins on the Surface of Mouse Preimplantation Embryos

Protein or reagent in culture media	Primary antibody	Secondary antibody	Results[a]			
			Embryos			
			2–4 cell (140)[b]	Morula (140)	Blastocyst (140)	Unfertilized eggs (140)
Supernate from BSA/α-BSA reaction	α-BSA	R-GAR[c]	–	–	–	–
Supernate from BSIg/α-IgG reaction	α-BSIG	R-GAR	–	–	–	–
BSIG[d]	α-BSIG	R-GAR	+	++	++	+
BSA	α-BSIG	R-GAR	–	–	–	–
BSIgG	α-BSA	R-GAR	–	–	–	–
BSA	α-BSA	R-GAM[e]	±	±	±	±
BSIgG	α-BSIG	R-GAM	±	±	±	±

[a] +, positive fluorescence; –, negative fluorescence; ±, slight fluorescence attributed to background staining.
[b] Total numbers of oocytes/embryos assayed per "protein in culture."
[c] GAR, goat anti-rabbit immunoglobulins (IgG fraction).
[d] Bovine serum IgG (different batch), also from Cappel Laboratories.
[e] GAM, goat anti-mouse immunoglobulins (IgG fraction).

Localization of Serum Albumin

Oocytes and embryos failed to show any consistent fluoresence when bovine serum albumin (BSA) was the protein present in the culture medium and/or when rabbit antibody to BSA was used as the primary antibody (Fig. 1a, Table I). The supernate from the BSA/anti-BSA precipitation reaction also failed to label oocytes and embryos (Table II).

When human serum albumin (HSA) was the protein present in the culture medium, immunofluorescence assays indicated that HSA was adsorbed by oocytes and embryos, even after antibody to HSA was first absorbed with soluble HSA. This particular antibody to HSA, however, was obtained from Sigma Chemical Co. and was not affinity purified as were the other antibodies used in this study. Upon further absorption of this antibody with mouse liver powder (Cappel Laboratories, Downingtown, PA) to remove nonspecific xenogenic activity against mouse tissues that is frequently found with rabbit sera, this antibody to HSA no longer labeled embryos that had been preincubated in HSA (Fig. 1b). This observation indicated that HSA as well as BSA did not adsorb to the cell surfaces of mouse oocytes and preimplantation embryos.

Localization of Serum Immunoglobulins

In contrast to what was observed with BSA and HSA, bright immunologically specific fluoresence was observed when the oocytes and embryos were cultured in media containing bovine (Figs. 1c and d) or human (Figs. 1e and f) IgG as the protein source and then treated with the corresponding antibody. The reaction pattern varied from flourescent rims in most cases to patches and caps in a few others. This was true for all the stages tested, although em-

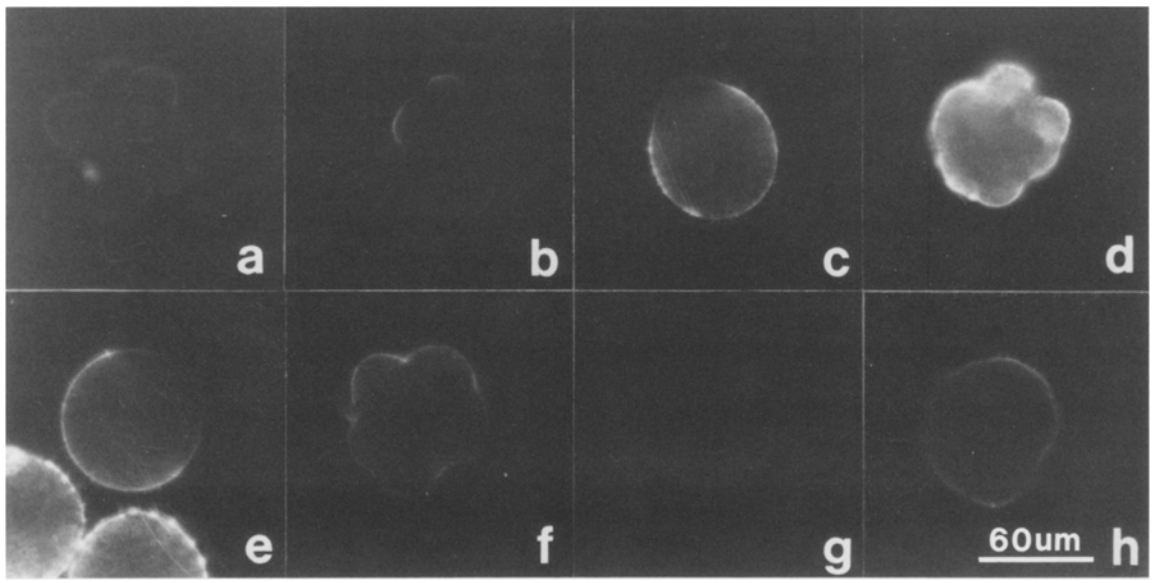

Fig. 1. Indirect immunofluorescence localization of serum proteins adsorbed to the cell surfaces of mouse oocytes and morulae. (a) Morula that was preincubated in bovine serum albumin (BSA) and then treated sequentially with rabbit anti-BSA IgG and rhodamine-conjugated goat antibody to rabbit Ig (Rh-GAR IgG). Note the absence of appreciable cell membrane fluorescence. (b) Morula that was preincubated in human serum albumin (HSA) and then sequentially treated with rabbit anti-HSA IgG and Rh-GAR IgG. Note the absence of appreciable cell membrane fluorescence. (c) Oocyte preincubated in bovine serum immunoglobulin (BSIG) and then treated sequentially with rabbit anti-BSIG IgG and Rh-GAR IgG. Note the cell membrane fluorescence. Fluorescence is absent on that region of the cell membrane overlying the spindle due to the lack of surface microvilli in this region (see Ref. 17). (d) Morula preincubated in BSIG and then treated sequentially as in c. Note the bright cell membrane fluorescence. (e) Oocyte preincubated in human serum immunoglobulins (HSIG) and then treated sequentially with rabbit anti-HSIG IgG and Rh-GAR IgG. Note the cell membrane fluorescence except for that region of the cell membrane overlying the spindle. (f) Morula preincubated in HSIG and then treated as in e. Note the moderate cell surface fluorescence. (g) Morula preincubated in HSIG, treated with rabbit anti-HSIG IgG that was exhaustively absorbed with HSIG, and then treated with Rh-GAR IgG. Note the reduced amount of cell surface fluorescence compared to the morula in f. (h) Morula preincubated in BSIG, treated with rabbit anti-BSIG IgG that was exhaustively absorbed with BSIG, and then treated with Rh-GAR IgG. Note the reduced amount of cell surface fluorescence compared to the morula in d.

bryos at the morula and blastocyst stages tended to show increased staining over those at the two- to four-cell stages. Slightly less fluoresence was observed when fetal calf serum was used as the protein source in the culture medium. For both human (Fig. 1g, Table II) and bovine IgG (Fig. 1h, Table II), the supernate from the IgG/anti-IgG exhaustive absorption precipitation reaction did not produce any fluoresence.

We also treated oocytes and embryos with goat antibody to mouse IgG to determine whether they could adsorb maternal IgG, to which they unavoidably become exposed during their removal from dissected oviducts. No mouse IgG was detected on these oocytes and embryos, in spite of the obvious presence of serum as evidenced by the formation of fibrin clots in the oviduct flushings.

Strain-Specific Effect

There was no notable strain-specific effect, as embryos from the three strains of mice tested showed a reaction pattern similar to that of the outbred mice; i.e., bovine serum IgG was bound preferentially over bovine serum albumin, regardless of the H-2 haplotype of the strain (Table III).

DISCUSSION

This study showed that mouse oocytes and preimplantation embryos are capable of adsorbing heterologous serum proteins—immunoglobulins in particular—from the culture medium. The adsorption of the immunoglobulins depended upon their species of origin, as human and bovine IgG were adsorbed while goat and mouse IgG were not. Adsorption per se was not dependent upon the strain of mice from which the embryos were obtained or upon the embryo stage, although there was an indication of increased adsorption at later stages (morulae and blastocysts) compared to earlier stages (two- to four-cell stage). Finally, neither human nor bovine serum albumin was adsorbed, even though their respective IgG's were specifically adsorbed by the oocytes and embryos.

The mechanism of this species-dependent adsorption of IgG is at best speculative. However, based on the specificity of the assay procedure used and the pattern of reactions observed, mouse preimplantation embryos and unfertilized eggs nonetheless appear to possess surface receptor molecules capable of recognizing serum IgG from certain animal species. The similarity of the results from the different strains tested suggest that these "receptors" are an integral part of the mouse genome and their cell surface expression and accessibility to immune antibody are not influenced by the H-2 haplotype.

The pattern of reactivity of the oocyte and embryonic cell surface with immunoglobulins of some, but not all, of the species is consistent with what would be observed for a heterophile determinant. Heterophile determinants comprise a group of cross-reacting antigens that are shared across species and phylogenetic lines and are of clinical importance in the host–parasite relationship (12). One such determinant is the "Forssman antigen," the presence and absence of which have been employed to classify different vertebrate animal species as being Forssman positive and Forssman negative, respectively (Table IV) (13). Those

Table III. Indirect Immunofluorescent Localization of BSIG and BSA on the Surface of Preimplantation Embryos of Mice of Different Strains

Mouse strain	Stage of embryo tested	Result	
		BSIG	BSA
C57B1/6J	2–4 cell (10)[a]	+	–
	Morula (10)	+ +	–
	Blastocyst (10)	+ +	–
BALB/cbjj	2–4 cell (10)	+	–
	Morula (10)	+ +	–
	Blastocyst (10)	+ +	–
CBA/HT6	2–4 cell (10)	+	–
	Morula (10)	+ +	–
	Blastocyst (10)	+ +	–

[a] Total number of embryos assayed.

Table IV. Presence or Absence of Forssman-Type Antigen in Red Cells of Some Common Animal Species[a]

Forssman positive	Forssman negative
Horse	Human[b]
Sheep[c]	Pig
Goat[c]	Rabbit[b]
Dog	Rat
Cat	Duck
Mouse[c]	Cattle[b]
Fowl	Deer
Hamster	

[a] Adapted from Ref. 13.
[b] IgG species that adsorb to mouse oocytes and preimplantation embryos.
[c] IgG of species that do not adsorb to mouse oocytes and preimplantation embryos.

species that are Forssman postive (i.e., goat, mouse) do not produce antibodies to Forssman antigen because they recognize such antigen as a "self" antigen. However, those species that are Forssman negative (human, rabbit, cattle) do produce antibodies to this antigen because it is perceived as "nonself."

Forssman antigen has been detected by monoclonal antibodies on mouse preimplantation embryos (14), and we found that IgG from human and bovine serum is adsorbed by such embryos. It is tempting to speculate that the IgG of those species that were adsorbed by oocytes and embryos is recognizing some heterophile Forssman-like antigen that is present on the surface mouse oocytes and all stages of mouse preimplantation embryos. If this speculation is cogent, then the prediction can be made that native IgG from Forssman-positive species such as the goat would not be adsorbed by mouse preimplantation embryos, while native IgG from the Forssman-negative rabbit would be adsorbed by such embryos (Table IV). This prediction is borne out in the literature. Neither normal goat serum nor IgG from normal goat serum labels mouse preimplantation embryos and both of these reagents are commonly used as "blocking" reagents in immunofluorescence assays where the second antibody is of goat origin (pretreatment serum; see Ref. 15). On the other hand, normal rabbit serum is well known for containing xenogeneic anti-mouse IgG that produces high background levels of labeling when used on mouse tissues during immunofluorescence antibody localization experiments. Incidently, the observation that not all species of IgG were adsorbed diminishes the possibility that Fc receptors on the oocytes and embryos were responsible for the observed adsorption of human and bovine IgG. Furthermore, we could find no evidence in the literature for the presence of Fc receptors on mouse oocytes and preimplantation embryos.

As stated in the Introduction, one reason for performing this study was to obtain information on why mouse preimplantation development in vitro is enhanced when the protein supplement consists of a mixture of serum IgG and albumin. At this time, however, the results obtained in the present study do not indicate whether the adsorption of BSIG and HSIG—but not BSA or HSA—is causally related to the culture data we obtained earlier (6), particularly in view of the observation that not all species of IgG adsorb to the embryos. In addition, goat IgG supports mouse embryo development in vitro as well as bovine and human IgG, even though goat IgG is not adsorbed by mouse preimplantation embryos.

The possible presence of Forssman-like antigens and possibly other heterophile antigens on mammalian gametes and early embryos is relevant to the choice of protein supplement for human embryo culture. As mentioned earlier, there is evidence that whole serum can be detrimental to human embryos in vitro. Some lots of human newborn cord serum have been reported to contain complement-dependent cytotoxic antibodies that can lyse the trophoblast of mouse blastocysts (16). However, it would be advantageous to obtain serum proteins from human sources rather than from nonhuman sources for use as a protein supplement because human proteins would not be expected to provoke an immune reaction if incidentally introduced into the mother during embryo transfer.

The above-mentioned antitrophoblast antibodies in female serum (16) raises another possible explanation for the observed adsorption of human IgG to mouse oocytes and early embryos. We were informed by Miles Laboratories—the vendor for the human IgG used in our study—that the source for their purified human IgG was pooled serum drawn from men and women. Consequently, there is the possibility that some of this serum might have been obtained from multiparous women who had developed antitrophoblast antibodies that were responsible (in part, at least) for our observations. It might be advisable, therefore, to screen commercially obtained human IgG for the presence of antitrophoblast and antiembryo antibodies prior to considering its application to IVF-ET.

Another factor that should be considered here is our observation that the zona pellucida was "labeled," regardless of the species of albumin or immunoglobulin to which the embryos were exposed. We have found that a 15- to 20-min rinse will remove such labeling from zonae—but not specific labeling from cell surfaces—and therefore feel that it represents nonspecific entrapment of protein in the meshwork of the zona. However, if nonhuman proteins were to be similarly entrapped by human zonae, then they could be incidentally transferred to the uterus because embryos are normally taken from the culture medium and placed directly into the transfer medium and then immediately placed into the uterus.

There is growing concern about the possible

dangers of AIDS and hepatitis transmission from the use of human serum or serum proteins in IVF-ET. This concern is particularly worrisome in cases where the serum/serum proteins are not from the same patient as the ova to be fertilized in vitro. The use of nonhuman serum or serum proteins to culture human embryos could reduce the risk of exposure to AIDS and hepatitis B but poses the danger of inducing immune reactions in the mother, especially if those proteins are adsorbed to the surface of the embryo during culture as we observed in this study. Consequently, if nonhuman serum proteins are to be used as protein supplements in culture media used in human IVF-ET, care should be taken to determine that those serum proteins are not adsorbed by human embryos. To our knowledge, no one has yet determined whether bovine, human, or other species of serum proteins adsorb to human embryos. Such determinations might, perhaps, be performed with polyspermic human ova or with ova that fail to fertilize. Finally, adsorption of human IgG by mouse oocytes and embryos also suggests caution in interpreting immunologic phenomena mediated by human sera against mouse embryos in vitro.

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REFERENCES

1. Shirley B, Wortham JW, Witmyer J, Condon-Mahony M, Fort G: Effects of human serum and plasma on development of mouse embryos in culture media. Fertil Steril 1985;43: 129–134
2. Menezo Y, Testart J, Perrone D: Serum is not necessary in human in vitro fertilization, early embryo culture, and transfer. Fertil Steril 1984;42:750–755
3. Caro CM, Trounson A: The effect of protein on preimplantation mouse embryo development in vitro. J Vitro Fert Embryo Transfer 1984;1:183–187
4. Cheung SW, Strickler RC, Yang VC, deVera M, Spitznagal EL: A mouse embryo culture system for quality control testing of human in vitro fertilization and embryo transfer media and fetal cord sera. Gamete Res 1985;11:411–419
5. Ackerman SB, Swanson RJ, Stokes GK, Veeck LL: Culture of mouse preimplantation embryos as a quality control assay for human in vitro fertilization. Gamete Res 1984;9:145–152
6. Wiley LM, Yamami S, Van Muyden D: Effect of potassium concentration, type of protein supplement, and embryo density on mouse preimplantation development *in vitro*. Fertil Steril 1986;45:111–119
7. Goldbard SB, Verbanac KM, Warner CM: Genetic analysis of H-2 linked gene(s) affecting early mouse embryo development. J Immunogen 1982;9:77–82
8. McLaren A, Bowman P: Genetic effects on the timing of early development in the mouse. J Embryo Exp Morphol 1973;30:491–498
9. Goldstein LS, Spindle AI, Pedersen RA: X-Ray sensitivity of the preimplantation mouse embryo *in vitro*. Radiat Res 1975;62:276–287
10. Quinn P, Barros C, Whittingham DG: Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. J Reprod Fertil 1982;66:161–168
11. Mintz B: Experimental study of the developing mammalian egg: Removal of the zona pellucida. Science 1962;138:594–595
12. Jenkin CR: Heterophile antigens and their significance in the host-parasite relationship. *In* Advances in Immunology FJ Dixon Jr, JH Humphrey (eds). New York, Academic Press, 1963, p 351
13. Boyd WC: Fundamentals of Immunology, 4th ed. New York, London, Sydney, Wiley Interscience, 1966, p 195
14. Willison KR, Stern PL: Expression of a Forssman antigenic specificity in the preimplantation mouse embryo. Cell 1978;14:785–793
15. Sternberger LA: Immunocytochemistry, 2nd ed. New York, John Wiley & Sons, 1979, p 95
16. Bronson R, Cooper G, Rosenfeld D: Anti-trophoblast complement-dependent cytotoxicity in normal human umbilical cord serum. Fertil Steril (abstr);1985:American Fertility Society Meeting, Chicago
17. Johnson MH, Eager D, Muggleton-Harris AL: Mosaicism in organization of concanavalin A receptors on surface membrane of mouse egg. Nature 1975;257:321–322