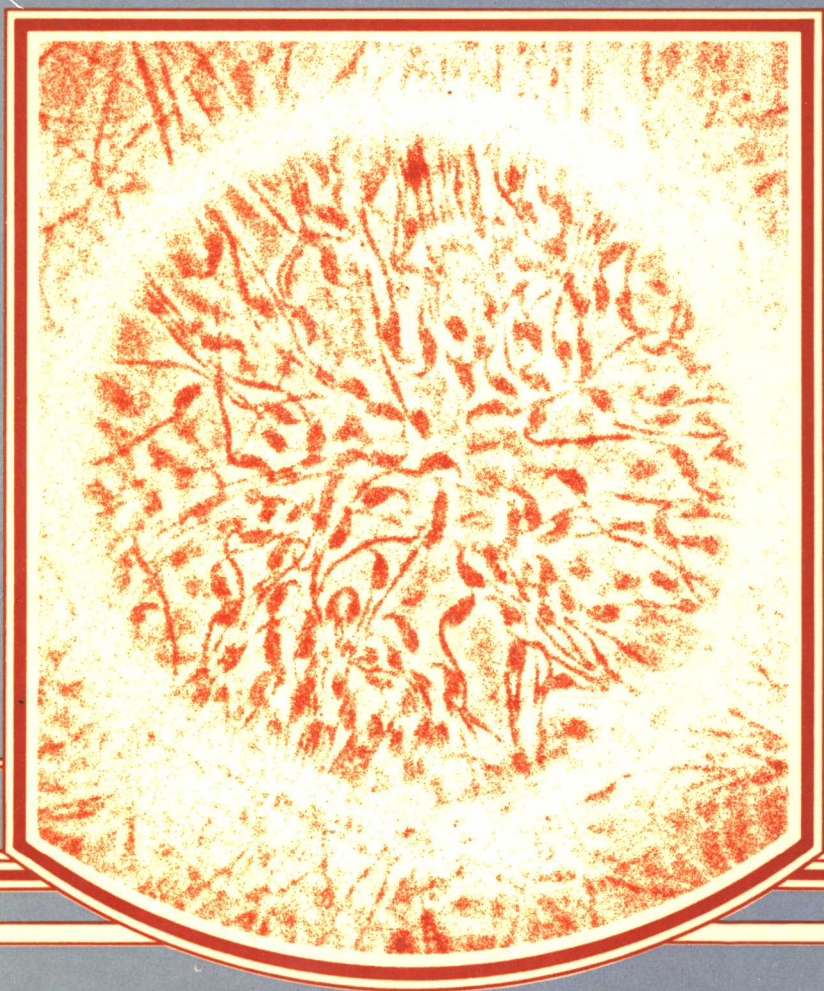


Immunological Aspects of Reproduction and Fertility Control

Edited by J. P. Hearn



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J. P. HEARN

Director

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The Zoological Society of London*




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Preface

The no-man's-land between reproductive physiology and immunology is becoming crowded. The last 10 years have seen a revolution in our understanding of many reproductive processes, brought about by the application of ever more sophisticated immunological methods. The increasing precision of these techniques has given us specific ways of assaying, enhancing or blocking hormonal mechanisms to yield more critical and interpretable information.

In this volume eleven authors have presented the current status and future prospects of some immunological aspects of reproduction and fertility control. These include the relationships between mother and fetus, the diagnosis of pregnancy, the immunological complications seen in clinical management of human reproduction and some novel approaches for immunological control of fertility.

We hope that in these chapters we have achieved an up-to-date account of a fast-moving field that calls on several disciplines. We intend the book to provide an adequate background and a current review for research workers and clinicians who wish both to understand the complex mechanisms involved and to develop improved scientific and clinical methods. We hope too that the student and newcomer will find this a useful reference book.

As editor I have tried to leave each chapter able to stand on its own, risking slight overlap in one or two places where the same topic has been approached from different angles. My thanks in full measure are due to the authors for their hard work and promptness, to Annabel Gomm and Connie Nutkins for their help with organization and to the publishers; particularly Harry Bracken, for their unfailing encouragement and support.

John Hearn
London, April 1980

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Section I

Immunological

Aspects of

Reproduction

1

Immunological Relationships between Mother and Fetus during Pregnancy

M. KAYE

THE PHYLOGENY OF PLACENTATION

Placentation is defined as 'any intimate apposition or fusion of the fetal organs to the maternal or paternal tissues for physiological exchange'¹. It is widespread amongst the invertebrates and vertebrates, having arisen independently in those phyla which exhibit viviparity, and has probably evolved each time from external fertilization to internal fertilization and oviparity, through ovoviviparity to viviparity.

The transition from oviparity to viviparity has involved considerably greater change in the reproductive functions of the female than the male. The trend of viviparity has been to optimize the potential of each individual egg, with a reduction in the number of eggs released. The yolk sac has been utilized for the absorption of nutrients from the uterus in vertebrates and the genital ducts have become modified to retain the embryo to an advanced stage. The synchronization of egg release with intromission and subsequent preparation of the genital tract for reception of the zygote has led to a refinement in endocrine control to synchronize mating, fertilization, pregnancy, parturition and, in the mammals, lactation. Such orchestration probably began in the specialization of a neural gland which by its proximity to the central ganglia would be in close contact with the environment through specialized sensory organs. In the phylum Chordata such a primitive pituitary gland exists in the Tunicata (sessile sea-squirts) which co-ordinates reproductive activity by

perceiving the sex products (pheromones) of other individuals in the water nearby².

In vertebrates, the reproductive functions of the maternal pituitary, ovary and corpus luteum have been extended not by the production of new hormones, but by the uses to which they are put³. In the mammals, 'maternal recognition' of pregnancy results in a dialogue between blastocyst and uterine epithelium, either directly or mediated via the corpus luteum. The mammalian feto-placental unit has come to intrude into the maternal endocrine environment to the point where it controls its own destiny from early in gestation so that ablation of the maternal pituitary or ovaries has no effect on gestation following implantation⁴.

Invertebrate placentation

Viviparity in the invertebrates is widely distributed, having arisen many times independently. A wide range of embryonic and maternal structures have been modified to assist embryonic nutrition. Viviparity occurs in the Platyhelminthes, Nermertea, Annelida, Onychophora, Insecta, Arachnida and Tunicata, whilst placentation is best seen in the Dermaptera, Ascidiacea, Thaliacea and the neo-tropical Onychophora. In the insects, the pseudo-placental Dermaptera, *Hemimerus talpoides* (earwigs), a few large embryos are held in the reproductive tract for long periods relative to the maternal life span. The embryo develops within the follicle surrounded by a maternally derived tunica propria. The anterior (containing the corpus luteum) and posterior pseudoplacenta develop from the follicular cells surrounding the ovum; these two maternal structures later degenerate to be replaced by two fetal placentas derived from the amnion. The embryos at birth are large and well-developed, but the function of the corpus luteum, present throughout gestation, remains unknown⁵.

Within the Tunicata, a compound ascidian displays many of the adaptations seen in the mammals. The ovary of these hermaphrodite sea-squirts produces a single small egg, devoid of yolk. The extra-embryonic membranes envelop the embryo and form an avascular placenta closely applied to the lining of the brood pouch which survives some 5 months and results in the birth of a large complex tadpole. The reproductive similarities between this lowly chordate and mammals demonstrates interesting parallels in functional adaptation⁶. Some species of the tunicate salpidae also develop an embryonal placenta which is intimately applied to the maternal surface and is similar to the placental attachment in the sea-squirt⁷.

The neo-tropical Onychophora of Trinidad forms a placental closely analogous to the yolk sac placenta of mammals (Figures 1.1 and 1.2). The embryo remains attached to its placenta for several months before detaching from the well-developed fetal-maternal placental zone to lie free in the oviduct where nourishment of the embryo continues from oviduct fluids prior to birth⁸.

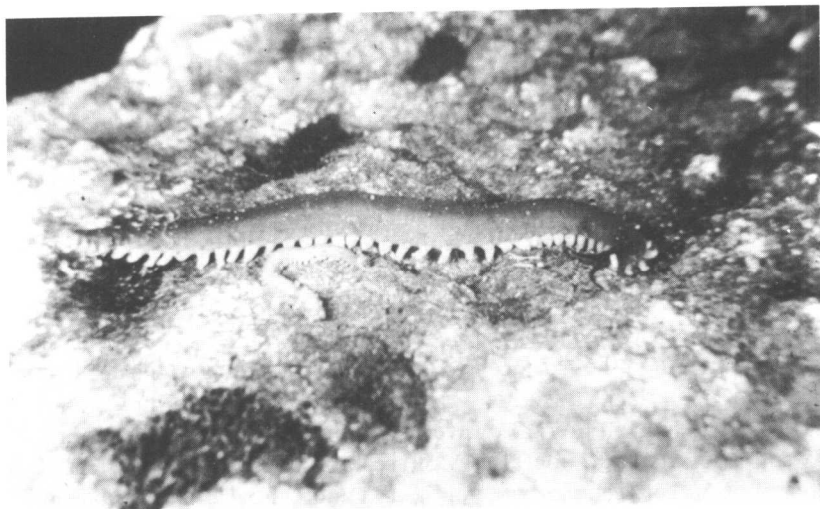


Figure 1.1 Mother and newborn *Epiperipatus* (65% of actual size). With kind permission of D. T. Anderson

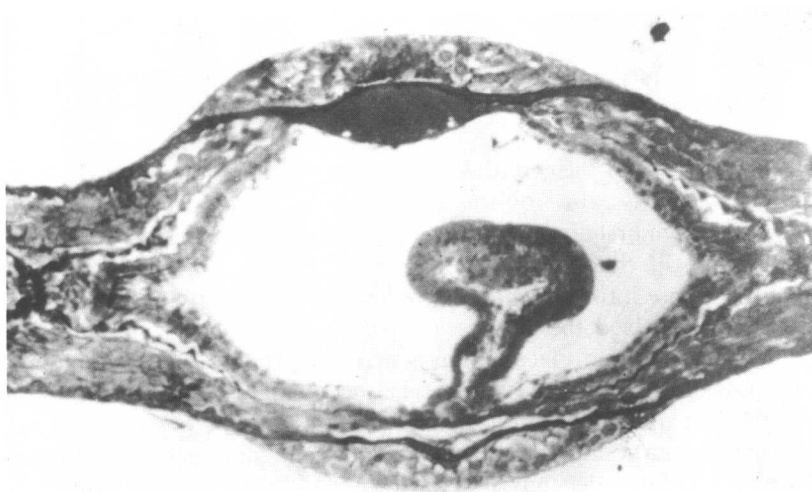


Figure 1.2 Longitudinal section through part of the oviduct of *E. trinidadensis* containing a stalked vesicle ($\times 130$). With kind permission of D. T. Anderson

INVERTEBRATE IMMUNE RESPONSES

Although the protozoa display cell-surface macromolecules demonstrable in mating, alloincompatibility appears to be intracellular⁹. The metazoa, however, recognize differences at the cell surface and all invertebrates from

Porifera (sponges) onwards demonstrate extensive allogeneic polymorphism and discriminatory immunorecognition. The tropical sponges display an increasing rate of reaction to first-, second- and third-set grafts which is both discriminatory and displays a specific memory¹⁰. Among coelenterates, transplacentation reactions vary from non-fusion to acute cytotoxicity; the effector phase may be non-specific but recognition is specific. The corals display memory with first- and second-set reactions to grafting, and demonstrate extensive polymorphism of histocompatibility antigens. Transplantation reactions are mediated by leukocytes in the annelids, which show differing cell types for immunorecognition and cell-mediated cytotoxicity functions. The arthropod immunological status is somewhat ambiguous. Non-self recognition is apparent in the crustacean decapods, while insects which encapsulate xenografts may lack a subtlety in their leukocyte immunorecognition response. The hymenoptera, however, show no such lack of discrimination between isografts and allografts. Molluscan reactions are characterized by differentiation between isografts, allografts and xenografts but quantitative studies remain unknown. Memory in the form of first- and second-set reactions to allografts is apparent in the echinoderms, perhaps mediated by leukocytes. Amongst the chordates the solitary and colonial tunicates exhibit allograft reactions, again possibly mediated by leukocytes. Fusion experiments have revealed extensive histocompatibility polymorphism. Thus it is clear that a refined immunorecognition based on polymorphism of cell-surface histocompatibility markers has originated in the earliest multicellular sponges or coelenterates¹¹.

The importance of the evolution of placentation and immune response in the invertebrates is raised by the absence of an humoral immunoglobulin system^{12, 13} which first appears in the most primitive vertebrate, the Agnatha or jawless fishes¹⁴. Since placentation probably evolved following the cellular immune response it follows that the change from oviparity to viviparity and placentation involved the embryo's avoidance of maternal immunorecognition. To suggest that placentation is dependent upon a specific immunological unresponsiveness of the mother mediated by an elegant humoral antibody system is irrelevant in the invertebrates, and perhaps equally so in vertebrates¹⁵. The ability of foreign fetal tissues and maternal tissues to reside in harmony probably depends on some form of cellular communication at the interface between the two organisms.

VERTEBRATE PLACENTATION

All the vertebrate classes exhibit placentation with the exception of aves. The placental attachment, as in invertebrates, represents an amazing diversity of adaptations of embryonal structures. The yolk sac placenta of the Chondrichthyes (cartilaginous fish) was first described by Aristotle *circa* 340 BC¹⁶.

In the Poeciliidae of the teleosts (bony fish) placentation is the result of extensions of the hind gut or pericardial sac of the embryo to the ovarian follicle in which gestation occurs. Embryos removed from the ovarian follicle and separated from their fertilization membrane (a product of the maternally derived vitelline membrane) and transferred to the peritoneal cavity of adults were destroyed by day 14 as allografts. Embryos transplanted similarly, but within their fertilization membrane, survived, emphasizing the importance of the maternal egg membranes in protecting the 'foreign' embryo for a gestation period of 28 days¹⁷. In other groups of teleosts, the *Jensynsii*, the embryo develops in the ovarian cavity, and placentation results from a fold of ovarian tissue coming into contact with the gills of the embryo. Both maternal and fetal circulations are separated by a thin layer between ovarian and gill tissues.

The amphibians, like the fish, are mostly oviparous; however, some have adapted to terrestrial life by having the usually aquatic larval stage maintained in the maternal host. In *Salamandra atra* the embryos, having exhausted their yolk sac supply, cannibalize other embryos in the uterus and feed on the blood from uterine haemorrhages. In the Surinam toad, the externally fertilized eggs develop in skin pouches on the mother's back.

All the reproductive structures of mammals are found in the reptiles; the cleidoic egg and the extra-embryonic membranes (amnion, chorion and allantois) are present in the oviparous reptiles. The latter have been adapted to form either a vascularized yolk sac or allantoic placenta in the viviparous snakes and lizards. Perhaps an example of the advantages of placentation over oviparity in related groups of snakes and lizards is the changing ratio of oviparity to viviparity with a changing environment. From sea level to 4000 feet, oviparity is the major form of reproduction; above 4000 feet viviparity begins to predominate. Similarly, as one journeys west from the south-eastern coast of Australia to the inland plains, oviparity gives way to viviparity¹⁸. The role of progesterone produced by the corpus luteum of pregnancy in placental reptiles remains enigmatic¹⁹. For an extensive review of the evolution of placentation the reader is referred to references 4, 20 and 21.

The vertebrate egg is surrounded by a primary membrane (vitelline membrane) a product of the egg itself, and a secondary membrane (zona pellucida) secreted by the ovarian follicular cells. The tertiary membranes are products of the oviducts (mucoid coat) and uterus (shell membrane); it is well developed in monotremes but is ruptured in the last third of gestation in mammals and is absent in eutherians²². These egg membranes play important roles in sperm-egg recognition and may prevent recognition of the foreign zygote during its sojourn down the female genital tract.

The extra-embryonic membranes of the amniote egg, the yolk sac for nutrition and the allantois with its separate circulation for gaseous exchange and repository of embryonic excretory products, have been adapted in viviparous vertebrates to varying degrees as placental attachments. In the change from oviparity through ovoviviparity to viviparity, the yolk sac

contents are reduced as the maternal-embryonic relationship becomes more intimate for the purposes of embryonic nutrition. The placental associations of the vascularized yolk sac and allantois with the non-vascular chorion (trophoblast) vary in their contribution to the definitive placenta, and their role in nutrition and gaseous exchange. In reptiles either the yolk sac or allantois takes part in nutrition and placentaion¹⁹, in marsupials the yolk sac usually forms the placenta, except in Peramelidae (bandicoots), where the allantois contributes²³.

In eutherians the yolk sac and allantois make varying contributions to the placenta. The yolk sac in the rabbit survives briefly; in the horse it survives to late gestation, and in the rat and squirrel it survives throughout gestation along with the allantoic placenta^{4,24}. The ability of some marsupials²⁵ and eutherian⁴ chorioallantoic placenta to invade uterine tissues appears unique, while the loss of chorionic ectoderm in the allantoic placenta of viviparous lizards appears to be the result of degeneration²⁶. The three forms of chorioallantoic placenta based on the differing nature of the fetal-maternal interface in reptiles²⁷, marsupials and eutherians has arisen independently in the three groups. The eutherian placenta may have evolved independently of the marsupial from a common oviparous ancestor some 150 million years ago²⁸, or from a common ancestor which was viviparous and nidiculous (young, helpless at birth and requiring extensive maternal care) with the marsupial having remained in the early state while the mammals evolved a longer gestation with a more mature embryo at birth²⁹.

VERTEBRATE IMMUNE RESPONSES

The evolution of the vertebrates has resulted in the continuing refinement of immune responses with the development of immunoglobulins and specialized lymphoid tissues. The thymus first appears as diffuse lymphoid tissue in the gill region of the Agnathan larval lamprey and is present in all other vertebrates. It develops early in ontogeny and provides the microenvironment for the bone-marrow lymphocytes to recognize self-antigens³⁰. The secondary lymphoid organs, spleen and lymph nodes, develop later in ontogeny and unlike the thymus persist throughout adult life. The haemopoietic foci of the anterior gut of the Agnatha probably represents a primitive spleen which is present in all vertebrates. The lamina propria of the gut contain lymphocytes from the Agnatha to mammals whilst lymph nodes and lymphopoietic bone marrow emerge at the amphibian anuran level to become highly structured in the marsupials and eutherians. The source of immunoglobulin-producing B cells, which arise from the cloacal bursa of Fabricius of birds, has a possible equivalent in the fetal liver or Peyer's patches of mammals; no avian bursal equivalent has been found in other vertebrates despite the presence of B cells³¹.

It is in the agnathan hagfish and lamprey that immunoglobulin-producing

cells first appear with IgM synthesis; the Osteichthyes (bony fish), amphibians and reptiles produce both IgM and IgG immunoglobulins whilst the eutherians synthesize some five classes (IgM, IgG, IgA, IgD and IgE)¹³. All vertebrates display transplantation reactions and memory, and possess lymphocytes which are the functional equivalents of thymic T cells and 'bursal' immunoglobulin-secreting B cells³¹. The process of collaboration which occurs between these cells is presaged in the annelids and results in an efficiency of immune reactions which is dependent upon shared haplotypes³². The perfection of this co-operation between helper and suppressor T cells, B cells and macrophages seen in the mammals may be seen to some degree in all the vertebrate classes with the exception of the Dipnoi (lungfish)³¹.

The presence of IgG antigen receptors both on B cells and T cells has been described in the Chondrichthyes, teleosts, amphibians and eutherians³¹. There is, however, a difference in the manner in which mammalian T and B cells 'see' antigens. B cells 'see' the antigen with that part of the surface immunoglobulin known as the variable (V) region, whilst T cells 'see' the antigen only in association with the appropriate syngeneic major histocompatibility determinant³³.

Table 1.1 Phylogeny of the immune system

	<i>Thymus</i>	<i>Spleen</i>	<i>Gut-associated lymphoid tissue</i>	<i>Peripheral lymph nodes</i>	<i>Allograft rejection</i>	<i>Serum antibody class</i>
Invertebrates	—	—	—	—	+	—
Vertebrates		(larval lamprey)				
Agnatha	±	±	+	—	+	IgM
Chondrichthyes	+	+	+	—	+	IgM
Osteichthyes	+	+	+	—	+	IgM, IgG
Teleosts	+	+	+	—	+	IgM, IgG
Amphibians	+	+	+	+	+	IgM, IgG
Reptiles	+	+	+	+	+	IgM, IgG
Birds	+	+	+	+	+	IgM, IgG, IgA
Mammals	+	+	+	+	+	IgM, IgG, IgA, IgD, IgE

± Primitive

* Germinal centres

SPERMATOZOA AND UTERINE RESPONSES IN MAMMALS

The presence of the major histocompatibility antigens on mammalian spermatozoa³⁴ and the local immunological uterine responses following coitus in the rat³⁵ pose interesting questions for reproductive immunobiology. The ability of the rat uterus to respond to the intra-uterine inoculation of washed epididymal spermatozoa by hypertrophy of the draining para-aortic lymph

nodes and transplantation immunity is in contrast to the inability of spermatozoa following coitus or pregnancy to elicit transplantation immunity despite similar hypertrophy of the para-aortic nodes. Pre-sensitization of one uterus by epididymal spermatozoa or leukocytes results in a 'local' reaction following rechallenge with the inoculum, but not when injected into the lumen of the non-sensitized contralateral uterine horn. The 'sensitized' uterus appears to gain enhanced reproductive performance over its non-sensitized homologue on an immunological basis³⁶.

The 'selection' of spermatozoa by the female mammalian genital tract results in only 5% of sperm reaching the oviduct. In rabbits there are two waves of sperm migration following coitus; the first is transferred within minutes to the peritoneal cavity prior to ovulation and these are non-motile. The second wave is held in the lower isthmus; these then migrate *en masse* to the ampulla following ovulation³⁷. The probability that uterine 'selection' of spermatozoa may have an immunological basis is strengthened by the observation that sperm recovered from the uterus in the mouse and rabbit have antibodies attached, whilst those in the oviduct do not³⁸. The inhospitable environment of the uterus to foreign spermatozoa may have resulted in the necessity for large numbers of sperm produced in animals. The previous suggestion that many spermatozoa are incapable of fertilization due to errors of chiasmata formation during spermatogenesis³⁹ has recently been refuted⁴⁰.

How spermatozoa survive long periods in the female genital tract of invertebrates or vertebrates remains unknown. The phenomenon is found in many of the vertebrates with storage occurring in any part of the genital tract from the cloaca to the ovary⁴¹. In the Chiroptera (bats) sperm storage may last for as long as 198 days before fertilization. The spermatozoa are occasionally found in the cells of the uterus but leukocytes have not been seen in the uterus despite the presence of the foreign spermatozoa⁴².

FERTILIZATION

The evolution of species depends on reproductive isolating mechanisms to inhibit gene exchange and subsequent hybridization between populations. The prezygotic barriers are: (1) ecological or habitat isolation; (2) seasonal or temporal isolation; (3) ethological or sexual isolation; (4) mechanical isolation; and (5) gametic isolation⁴³. It is (5) which, both in the past and the present, is important for the mutual recognition of male or female gametes released either into the air or the water, in those life forms exhibiting external fertilization, which prevents interspecific hybridization. This recognition at fertilization exists from seaweeds⁴⁴ to animals whose fertilization either is external or internal⁴⁵.

The unfertilized egg releases soluble products which have four varying effects on spermatozoa as they approach:

- (1) Increased sperm motility.
- (2) Chemotaxis, which is incompletely species-specific and is present in coelenterates, urochordates and possibly other animals⁴⁶.
- (3) Sperm agglutination, which in a variety of animal species occurs in the presence of fertilizin (egg supernatant glycoprotein); it is species-specific and thought to be the initiator of the sperm acrosome reaction. The occurrence of sperm agglutination by egg supernatant has been described in molluscs, annelids, echinoderms, chordates and plants⁴⁵.
- (4) The sperm acrosome reaction; beneath the plasma membrane of the sperm lies the acrosome containing hydrolytic enzymes which when released are thought to aid in sperm entry through the egg envelopes⁴⁷, together with the slicing action of the sperm head due to tail movement⁴⁸.

Recognition in sea urchins is dependent upon a glycoprotein on the egg vitelline layer (analogous to the zona pellucida of mammals) to which the sperm attaches⁴⁹; this species-specific interaction is probably a generalized phenomenon with the receptors in the mammalian egg residing on the zona pellucida. The recognition site in mammalian spermatozoa is the inner acrosomal membrane which is uncovered following the acrosomal reaction prior to fertilization. Fusion with the microvillous areas of the egg plasma membrane occurs in the post-acrosomal region of the mammalian spermatozoa, in contrast to invertebrates where fusion of the spermatozoa and egg plasma membrane takes place at the inner acrosomal membrane. In some mammals the reaction at the zona pellucida prevents the penetration of heterologous spermatozoa; however, zona-free eggs of the hamster may be penetrated by the spermatozoa of a number of mammalian species⁴⁸.

The entry of the spermatozoa into the egg of the sea urchin results in a depolarization of the egg surface membrane within 3 s and an immediate partial block to further sperm entry. Some 30 s later the cortical granules evacuate into the perivitelline space forming the fertilization membrane which carries away from the egg surface all other unsuccessful sperm, and prevents polyspermy. The fertilizing sperm, now immotile, is engulfed by the egg cytoplasm⁵⁰.

In mammals there may also be two blocks to polyspermy. In the rabbit egg there is a cortical vesicle reaction, but no zonal reaction, the egg plasma membrane surface receptors to spermatozoa are probably lost. Other mammals have a zona reaction following the cortical reaction with perhaps loss of sperm receptors from the zona⁵¹. It would not be idle speculation to wonder if this loss of receptors from the zona following fertilization is responsible for the ability of transferred eggs to survive in non-related recipients prior to the development of the trophoblast.

The recognition molecules which exist for successful species-specific mating