Section of Obstetrics & Gynæcology

President Robert Percival FRCOG

Meeting 23 November 1973

Scientific and Clinical Aspects of Fertilization and Implantation

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Principles of Fertilization

Fertilization consists of two major events – the coming together or approximation of the gametes and their fusion or union. The second of these may be subdivided into cytoplasmic union or plasmogamy, and nuclear union, karyogamy.

Approximation of Gametes

In animals with internal fertilization it is obviously necessary for the spermatozoa to be brought to the site of fertilization from the point at which they were deposited in the female tract at coitus, and their transport can represent quite a complex series of events.

In mammals, transport of spermatozoa is largely a function of the female tract, sperm motility playing generally a very minor role. In man, however, sperm motility is important in two locations: the cervix of the uterus and the immediate environment of the eggs. Owing to the high viscosity of the cervical mucus, coital activity does not seem to be responsible for more than a small advance of the semen into the cervical canal. The rest of the journey through the mucus plug has to be made by the spermatozoon by means of its own motility (*see* Moghissi 1971). Because of the orientation of the long glycoprotein molecules in the cervical mucus, spermatozoa tend to be directed towards the surface of mucous membrane lining the cervical canal (the site of mucus secretion) and come to rest here for variable periods of time. The circumstances are rather similar to those that pertain in the ruminants, in which sperm passage through the cervix has been studied in greater detail (Gibbons & Mattner 1971). In these animals it has been found that conditions within the mucus plug are highly favourable to sperm survival, and for this reason, and because spermatozoa tend to become retained against the mucous membrane, the cervical canal appears to function as a kind of reservoir from which spermatozoa may find their way to the site of fertilization some time, even many hours, after coitus.

Through the rest of the uterus and the oviducts the spermatozoa are transported chiefly by the muscular activity of the tract walls and the beating of cilia in the mucous lining. When the spermatozoa reach the site of fertilization, however, they meet the cumulus mass surrounding the eggs, and again depend upon their own motility to make their way through this investment. Both the cumulus matrix and the zona pellucida must be penetrated and this is evidently achieved through motility aided by enzymes from the sperm acrosome. Release of enzymes (hyaluronidase and a trypsin-like protease) depends first upon the occurrence of capacitation, which involves a change in the stability of sperm membranes, and this is followed by the acrosome reaction, wherein the membranes covering the anterior half of the sperm head become converted into vesicular structures. This results in the escape of the enzymes from the interior of the acrosome (Fig 1). The need for capacitation has

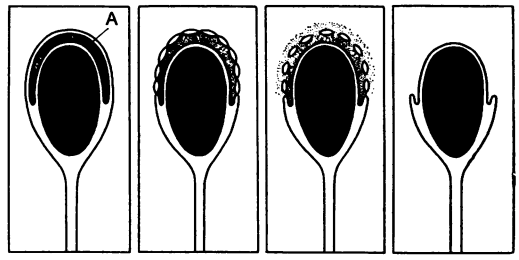

Fig 1 *Stages of the acrosome reaction, with vesiculation of membranes and release of the acrosomal enzymes.* A = *acrosome; solid black = nucleus.* (*Reproduced from Austin 1972 by kind permission*)

been accepted for more than twenty years but its mechanism still remains a mystery, and the time required for its completion varies considerably in different animals (Table 1). Despite ignorance of its nature, the conditions required for the occurrence of capacitation are sufficiently well understood to permit the *in vitro* fertilization of the eggs of several animals (*see* Table 2, which also indicates the stage of development achieved on subsequent culture, providing evidence of the viability of the zygotes). In the rabbit, hamster, mouse and rat, eggs fertilized *in vitro* have been transferred to host animals, in which they have succeeded in developing through to term; the young born have subsequently reached maturity and reproduced in their turn, thus demonstrating the normality of embryos arising in this way. With hamster eggs it has been possible to obtain fertilization *in vitro* in a fully-defined medium (Table 3); as yet the best results with the fertilization of human eggs *in vitro* have been reported with a medium consisting of a physiological saline solution to which are added sodium pyruvate and crystalline bovine serum albumin (Edwards 1973, 1974).

Table 1

Time required for capacitation of spermatozoa *in vivo*

Mammal	*Time (hours)*
Mouse	< 1
Sheep	1½
Rat	2–3
Hamster	2–4
Pig	3–6
Ferret	3½–11½
Rabbit	5
Rhesus monkey	5 or 6
Man	5 or 6

Table 2

Mammalian eggs fertilized *in vitro* (roughly in the order achieved)

Animal	*Source of spermatozoa*	*Stage reached with culture*
Rabbit	Uterus	Blastocyst
Golden hamster	Epididymis	2-cell
Mouse	Epididymis	Blastocyst
Chinese hamster	Epididymis	2-cell
Cat	Uterus	Cleavage
Man	Ejaculate	Blastocyst
Sheep	Uterus	Cleavage
Pig	Uterus	Cleavage
Guinea-pig	Epididymis	Pronuclei
Rat	Epididymis	Cleavage

Table 3

Medium of defined composition permitting fertilization of hamster eggs *in vitro*

(B D Bavister, personal communication, 1973)

Tyrode's solution
Glucose
Sodium pyruvate 0.5mM
Dextran 10 mg/ml
Penicillin
Osmolarity (mOsm/kg) 300 ●
pH 7.6

● Roughly that of serum

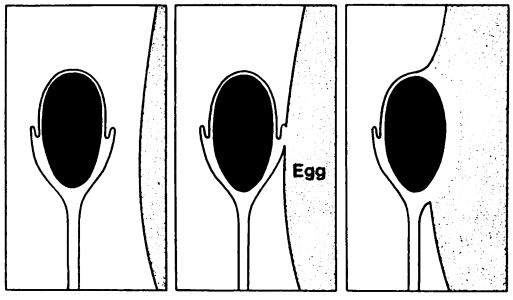

Fig 2 *Stages of fusion between spermatozoon and egg. (Reproduced from Austin 1972 by kind permission)*

Union of Gametes

Plasmogamy: Immediately upon passage through the zona pellucida the fertilizing spermatozoon makes contact with the vitellus and soon becomes attached to its surface. Attachment involves close approximation of sperm and egg cell membranes, and then fusion occurs (Fig 2). At this point the gamete membranes form a single continuum, so that spermatozoon and egg now, in fact, constitute a single cell. The area of fusion enlarges and egg cytoplasm flows around the sperm nucleus and gradually engulfs the tail.

Karyogamy: Once the sperm nucleus is suspended in egg cytoplasm it loses its nuclear envelope and begins to expand. Simultaneously the egg chromosomes left behind after the second meiotic division come together and also begin expansion. New nuclear envelopes form about both sperm head and egg chromosomes as they become transformed into vesicular pronuclei, which undergo considerable enlargement and finally assemble in the centre of the egg. Fertilization concludes with the disappearance of nuclear envelopes and a recondensation of chromosomes, which then aggregate in the prophase of the first cleavage division.

Significance of Fertilization

The two phases of gamete union have quite a different significance. Plasmogamy provokes the start of development; in most instances in mammals embryonic development does not begin unless and until sperm penetration takes place. As a rare spontaneous event or in consequence of experimental intervention, development may proceed without sperm penetration, and in this way parthenogenesis is initiated. There is as yet no certain record of the birth of a parthenogenetic mammal, but under experimental

Table 4

Comparison of meiosis and karyogamy

Meiosis	*Fertilization*
Crossing over + chromosome reduction → reassortment and elimination of genes	Karyogamy → recombination of genes
Promotes line uniformity but + mutation → new races	Promotes integration within race and adaptive evolution
Diploid → haploid	Haploid → diploid

conditions parthenogenetic mouse and rabbit embryos have proceeded apparently normally to about half-way through pregnancy before regressing. Plasmogamy thus has reproductive importance.

By contrast karyogamy has genetic importance. The full significance of karyogamy can only be properly appreciated when it is considered in relation to meiosis (Table 4). In the early stage of meiosis, crossing-over occurs between chromosomes of maternal and paternal origin, and thus new mixtures of genes are achieved; then, in the course of the two meiotic divisions the cell chromosome number is reduced to half, i.e. to the haploid state. In oogenesis half the chromosomes are eliminated in the polar bodies (for more details *see* Baker 1972). Consequently the major effects of meiosis are the reassortment and elimination of genes. In this way unfavourable genes can be jettisoned, a process of survival value for animal species living in highly stable and restricted environments, but meiosis alone would be of little benefit for adaptive evolution since no new genes are introduced into the race and innovation can only come by mutation. On the rare occasions when the mutation is favourable the consequence would be the establishment of essentially a new race. Thus meiosis alone results in rigid uniformity, and combined with mutation it can give rise to an increasing number of divergent races. Fertilization (specifically karyogamy) on the other hand, involves the recombination of genes from two different sources and as a result it promotes integration within the species as a whole, yet allowing variation and favouring adaptive evolution. This is not of course true in self-fertile hermaphrodites, as in certain fish, but does apply so far as we know to all mammals. Finally, fertilization is responsible for the restoration of diploidy.

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Recent Research on Implantation in Animals

Research on implantation has been carried out mostly on rodents, especially rats and mice. Because of ethical and technical difficulties very little work has been done on humans. Implantation in women is, in any case, a rather rare occurrence, although the preparation of the uterus for implantation occurs very regularly and is of considerable gynæcological significance. This paper concentrates on the work we have done on the preparation of the uterus for implantation in the mouse.

In all animals the ovum divides whilst passing down the oviduct and has developed to the blastocyst stage before arrival in the uterus, or soon afterwards. Initially, the trophoblast is pressed up against the microvilli of the uterine epithelium (Fig 1). At the time of implantation the luminal surface of the uterine epithelial cells undergoes a characteristic change, with the disappearance of the microvilli so that close contact is established between the epithelium and the surface of the

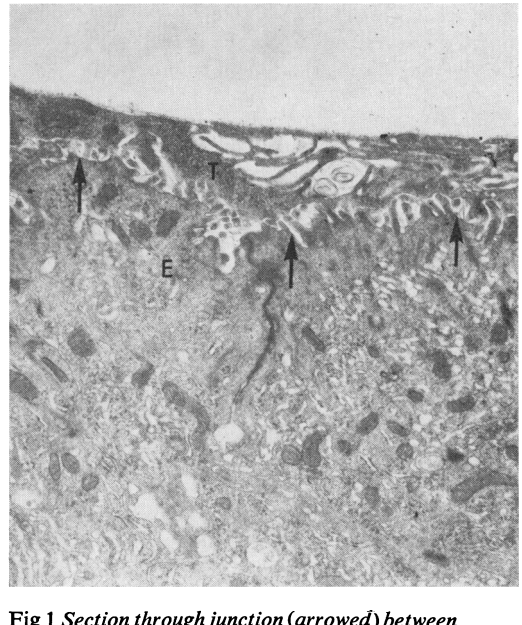

Fig 1 *Section through junction (arrowed) between trophoblast and uterine epithelium before attachment of blastocyst. The trophoblast is resting against the microvilli of the luminal surface of the epithelial cells.* (T, *trophoblast;* E, *epithelium*). (*By courtesy of Mrs J Downie*)