Review Article

Dilemmas of Early Human Life (Fertilization to Implantation)

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Remarkable insight into the mating dance of sperm and egg giving rise to human life have helped scientists to understand the various dilemma involved.

Testis continuously churn out sperm at the prodigious rate of 1000 a second (30 billion a year), the ovaries never produce new eggs. The eggs a woman is born with, usually about 2 million — are all she will ever have. By puberty, normal degeneration will have reduced the number to about 400,000. Although a woman may have 400,000 eggs to start with the number she can effectively use is closer to 400.

Spermatozoa once released at ejaculation are motile but they do not have fertilizing capacity. The three major physiological events in order of occurrence are capacitation, the acrosome reaction and hyperactivated motility. Capacitation occurs while sperm continue their migratory process within the uterus and fallopian tubes. Free calcium and sodium are key inducers. The acrosome reaction involves fusion and vesiculation of the outer acrosomal membrane and surrounding plasma membrane, leading to dispersal and release of acrosomal contents. (The acrosomal content mainly acrosin, which is essential for the fertilization process, is usually released only after binding of sperm to the zona pellucida).

Hyperactivated motility of sperm occurs after sperm binding to the zone, necessary for penetration of matrix of the zona.

Large number of sperms in the immediate vicinity of the oocytes with the hyaluronidase dissociates the cumulus matrix.

Fertilization : Timing of fertilization events

The process of fertilization in the human can be divided into three main stages (1) the attachment to and subsequent penetration of the zona pellucida by the spermatozoa (2) gamete fusion, sperm incorporation and activation of the oocytes (3) decondensation of the sperm nucleus and formation of the male and female pronuclei.

Normally fertilisation occurs in the ampulla of the Fallopian tube. The major barrier between the gametes is the zona pellucida. The zona has several important properties : it allows sperm penetration, but after the cortical reaction prevents polysperm penetration. All the cortical granules migrate to the side and they secrete certain enzymes which block the entry of more sperm — called the cortical reaction. The gamete fusion event initiates extrusion of cortical granule contents from the cortical surface of the oocyte into the perivitelline space. Cortical granule exocytosis occurs circumferentially and is completed within a few minutes. The cortical granules contains enzymes that interact with inner surface of the zone creating a dense inner structure that cannot be digested by sperm enzymes, preventing supernumary sperm penetration. A sperm to activate the oocyte has to release a cytosolic factor from its tail that diffuses into the ooplasm of the oocyte and subsequent hyperpolarization of the oocyte is seen. This phenomenon is known as activation. The activation

of the oocyte triggers a series of biochemical processes in the ooplasm that would eventually lead to sperm nuclear decondensation, second polar body emission, pronuclear formation. The sperm cytosolic factor has shown to be protein based. This protein is reported as oscillin. Oscillin is found in high concentrations in the intracellular part of the equatorial segment of the sperm. Oscillin triggers the intracellular calcium oscillations that eventually involve calcium release via channels in the endoplasmic reticulum.

When sperm fuses with the oocyte, a process akin to phagocytosis occurs where the midpiece of the sperm is engulfed by a microprocess extended by the oocyte cortical ooplasm, inner acrosomal membrane ruptures at several points when the sperm head is incorporated within the oocyte allowing chromatin decondensation to begin.

The oocyte is activated soon after the fusion of the gametes, with anaphase II of the second meiotic division and the second polar body is abstricted.

Nuclear membranes form around the decondensing sperm and oocyte chromatin and the pronuclei move into close proximity in the center of the oocyte within 3 – 6 hrs of gamete fusion where they remain for 20 — 24 hrs after insemination. Replication of DNA occurs at the pronuclear stage. Fully developed male pronuclei are first observed approximately 12 hrs after insemination.

During the first two cleavage division the embryo is reliant on maternal mRNA produced during oogenesis and the new embryonic transcripts are thought to be activated during the 4 -- cell stage of development. Hence arrest at four cell stage may occur after ET on Day 2 in which the embryo will be at four cell stage.

The oocyte is not simply a bag of cytoplasm with two nuclei. It is a most organised dynamic cell, highly polarised with some maternal mRNAs and proteins restricted to particular areas eg. a limited zone in cortex and which actually shift their position from one specific site to another as the oocyte matures. One consequence of this tight control is that the male pronucleus is four times more active transcriptionally than the female pronucleus. The difference between pronuclei could be crucial to the early regulation of the egg and any upsets in this difference between male and female pronuclei could impair its normal growth.

Genetic control of embryo quality

Genes inherited from the two parents may have influence over the embryo quality. These genes are Q7 & Q9. Fast embryos express either one, slow embryos express neither. This minor genetic change converts embryos from fast – to slow developers. The possibility of converting slow growing human embryos into those dividing more rapidly could improve implantation rates.

Implantation and early pregnancy

Implantation presents the greatest challenge to all forms of assisted human reproduction. Far less than one third of human embryos replaced in the uterus complete the initial stages of implantation. It is a period of life during which successful interactions between the embryo and uterus are imperative. This is a dilemma.

Due to hatching of the blastocyst on Day 7 the inner cell mass, trophoetoderm and blastocele cavity escape. Hatching is impaired if the zona pellucida has become hardened and this may have developed by the premature discharge of some cortical granules during oocyte maturation, or through the release of oximes and other free radicals from cumulus or granulosa cells.

The over exposure of oocytes to spermatozoa during fertilization in vitro may be another cause of zona hardening in human blastocysts. Embryos failing to hatch in vitro perish within their zona pellucida.

Blastocysts send signals to the mother which exert profound effects on maternal endocrinology and physiology. Human blastocyst secrete hCG in vitro, especially after hatching. Multiple factors are involved in initiating hCG secretion from human blastocyst by D 7 - 8 in vitro, including insulin, transferrin and platelet - derived growth factor (PDGF).

The embryo also prepares for its attachment to

the uterine epithelium. Cytotrophoblast, synctiotrophoblast and primitive endoderm appear on D 6-7. Trophoblast expresses many cytokines and other receptors on microvilli and gives the impression of awaiting implantation. Among these receptors, mRNA for the LIF (leukaemia inhibiting factor) receptor has been identified. This coincides with the expression of LIF in the maternal endometrium. These compounds may be involved in the initial attachment or approximation of the embryo to the uterine epithelium.

Implantation

Blastocysts can be divided as producers and nonproducers. Producers stimulate uterine epithelium to produce integrin and nonproducers do not.

Implantation begins as embryo becomes closely opposed to the uterus (apposition) and proceeds through adhesion and penetration. The initial phase involves the synthesis of adhesive glycoproteins by the epithelium and trophoblast. Specific substrates and receptors are expressed in the uterine epithelium decidua. Embryos produce enzymes including proteases which may help them to penetrate epithelial basement membranes. Collagenase released from the embryo might degrade uterine collagen. Prostaglandins released from the embryo might enhance uterine perfusion.

All the information through scientific work indicates a high degree of tissue organisation during the implantation of the embryo. Perhaps more than one biochemical system is involved in adhesion, penetration and other systems, to provide a "fail — safe" back up in case one of the systems should become ineffective.

Integrin expression in the uterus is highly regulated during implantation. The appearance and disappearance of two of the heterodimeric molecules frame the implantation window. $\alpha_v B_3$ (the vitronectin receptor) appears abruptly and reliably on Day 6 post ovulation. $\alpha_v B_1$ (a fibronectin receptor) appears just after ovulation and persists until Day 10. Their co-expression defines the implantation window, which closes as they disappear.

A defective expression of $\alpha_v B_3$ may be associated with infertility. A loss of B_3 (called type I infertility) involves out of phase endometrium, which is easily corrected by progesterone. Hence a_1 is active at ovulation and switched off at midcycle and B_3 is active at implantation.

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Uterine endometrium growth and implantation

Endometrium is thick and edematous as implantation approaches. Its epithelial surface has a mucus film and expresses microvilli and apical protrusions (Pinopodes). None are present on D 16 in natural and induced cycles, where 78% of human uteri on D 20 (D 16 after ovulation) possess their structures which persist through D 21 - 22. All of them are not seen by D 24 indicating they function only during implantation period. Pinopodes apparently absorb molecules and fluid from the lumen and so facilitate implantation by drawing the uterus closely around the embryo.

Extensive biochemical changes occur in epithelial cells preparing for implantation which may be deficient in infertility.

Dilemma of the Role of Progesterone in the implanting uterus:

Progesterone is the powerhouse of the luteal phase with many good effects like stimulation of stromal edema, stabilising lysosomes, immuno-suppression, lowering uterine irritability, stimulating formation of pinopodes etc. It might stimulate insulin like growth factor binding protein (IGFBP — 1) synthesis in the endometrium and regulate endometrial superoxide dismutase expression during stromal pre-decidualization. Luteal phase support could stimulate this metabolic process which might be unnecessary and even harmful. It may lower hCG production, thus explaining why this hormone plateaus during pregnancy. Progesterone inhibits the spontaneous pulsatile secretion of hCG by first trimester placental implants in vitro and some early pregnancy failure

may be due to its high levels in the mother's circulation.

Decidualisation and preparation of the uterus for implantation

This is essential for implantation. "True" decidualisation may begin only as embryos signal their presence to the uterine epithelium, or as activated macrophages release local factors as tumor necrosis factor. The decidua sustains the penetrating embryo during and after implantation by producing a matrix of substrates and adhesives and surface receptors for matrix proteins especially laminin. This occurs during the late luteal phase.

Laminin becomes dense and pericellular and has an important role in the adhesion of the embryo to the uterus. Fibronectin is an extracellular matrix protein and a major component of the decidua basement membrane. It is produced as stromal cells are exposed to progestins. Cells attach to fibronectin via the integrins and other receptors. Cells produce antigens CD 26+ which may be a marker for implantation.

Role of hCG

It is apparently essential to sustain early pregnancy. It supports the corpus luteum. When bound to trophoblast, it may stimulate adenyleyclase. It might be immunosuppressive at high concentrations. The output of hCG could be curtailed in various ways by slow embryonic growth, insufficient trophoblastic cells, lack of villous structure, failure of cytotroblast to penetrate into decidual cells and inadequate expressions of genes from hCG.

Adaptations in the embryo for uterine life

Embryonic adaptations for uterine existence has its haemoglobins getting adapted to combine with O_2 at very low tensions and pH and they disappear after 2 months. This switch can be arrested or may be abnormal under some circumstances. PO_2 is low in placental intervillous spaces and initially lower than in underlying endometrium, but it increases steadily from 6-14 weeks.

Local growth factors regulate early human placental and local transport systems are believed to carry specific metabolites to and from the endometrium. Several locally produced proteins may serve as carriers which sustain trophoblast.

Immune system

Several systems may protect the fetus from mother's immune responses to fetal antigens.

Conclusion

It is indeed a dilemma that a good number of anatomical, physiological biochemical and genetic changes are involved from fertilization to implantation which require good co-ordination for a better outcome towards normal pregnancy.