NEWS AND VIEWS

CONTROVERSIES IN ASSISTED REPRODUCTION AND GENETICS

The Uterus: Idiot Savant or Rosetta Stone?

AGING AND FECUNDITY

Marked, time-dependent decreases in pregnancy and implantation rates and a significant increase in the number of spontaneous abortions of chromosomally normal conceptuses characterize the onset and progression of reproductive aging in the female reproductive tract (1,2). Reproductive aging is a progressive decline in reproductive capacity that begins, in women, many years before absolute dysfunction, i.e., menopause. In terms of life span the female reproductive system ages precipitously. Compared with other major organ systems the female reproductive system devolves to complete failure at a relatively early age (3). The onset of decreasing reproductive capacity is followed sequentially by disruption of menstrual cyclicity, complete ovarian failure, and menopause. The cause for the onset and the progression of failure of the female reproductive system remains unexplained.

OOCYTE THEORY

Recent studies have accented the singular importance of oocyte quality (4) and age (5) in the outcome of pregnancy. Cast against a philosophy that perceived the maternal host as a passive, altru-

istic bystander that accommodates itself to every need of a self-preserving aggressive conceptus (6), a thesis has advanced the oocyte as the principal, if not the only, dominant element in the age-related increase in the number of spontaneous abortions of chromosomally normal conceptuses (2,3). This populist conclusion, that the only requirement for a woman to become pregnant (irrespective of fertility problems of the couple) is the uterus, derives from the compelling success of oocyte donation programs in normalizing the pregnancy rates of older women (>40 years) to that of younger women (<25 years) (7–9).

DOES THE UTERUS HAVE A PROACTIVE ROLE IN ESTABLISHING PREGNANCY?

Is the uterus, as the oocyte theory implies, indeed an idiot savant? Can mammalian reproduction be ex utero as described by Huxley's *Brave New World*? Although a significant penalty of the age-related decline in fertility (1) can be assigned to the oocyte (2,3), the inability to eliminate early preimplantation losses by the use of even the most sophisticated protocols for oocyte selection does suggest that aging effects (menarche, perimenopause) on pregnancy wastage (10) is also affected by the differentiated functions of the uterus (11). There is new evidence that on many levels of organization, i.e., cellular, tissue, and organ, the uterus plays a determinative role in the outcome of pregnancy.

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Oocytes from a single donor (young) transferred to recipients of different ages (21–49 years) produced relevant differences in pregnancy and implantation rates thereby implicating the uterus as a pivotal factor in pregnancy outcome (12). Asynchrony in the reproductive cycle alters the regulated program of differentiation of the endometrium. Whether these aberrations in programmed expressions will allow the uterus to accept (ovoreceptive) or not accept (nonreceptive) the developing blastocyst is cited as a principal causal event in pregnancy wastage. Our limited understanding of asynchrony/dysynchrony in the development and expression of an ovoreceptive endometrium may be the basis for the circumscribed success of hormone replacement therapy. That reproductive aging evolves before absolute dysfunction of the system suggests the possibility of correction.

UTERINE RECEPTIVITY

The maternal host is highly selective. It will discriminatingly permit interaction, i.e., attachment, of the developing embryo only within a stringently controlled (regulatory factors, structure, space, time) phase of endometrial cell growth and differentiation (13,14). For endometrial cells to develop and express their apparently independent but operationally interdependent role in establishing pregnancy and initiating and maintaining placentation (15,16) requires that the uterus become receptive, i.e., to allow and sustain adhesion by the developing embryo. To become receptive the endometrial cells must organize the elements that serve to develop a molecular vocabulary that provide the languages for cell–cell, cell–substrate, and tissue–tissue reciprocal communication. This dialogue is the signature of the evolving maternal–fetal interface (immunocompetence, growth, differentiation, scheduled invasion) (6,13,16).

CONCEPTUS-ENDOMETRIUM RECIPROCAL SIGNALING

Tolerance of the initial attachment of the embryo to the early secretory endometrial epithelial cell represents the symbiotic relationship between the conceptus and the maternal host. This intimate, complex relationship between two organisms of different genetic derivation (13,14) involves a devel-

oping network of reciprocally communicating systems that is the basis for the continuation of the species (6). The goal of these conversations is to secure the success of a program that directs the developing embryo to seek the site in the uterus most optimally prepared, structurally and functionally, to support the apposition and then the attachment of the blastocyst to the receptive endometrium.

If all elements are in order, these primary embryo–endometrium interactions can then initiate a program of sequential events, which, if correctly expressed temporally and spatially, assure the embryo of a secure place for nutrition, growth, and differentiation, e.g., development of a functional placenta (6,14,17). Because this communication network, which defines the symbiotic relationships between the conceptus (embryo, trophoblast) and the endometrium (epithelium, stroma), develops progressively on multiple functional and structural levels of biological organization, it depends on the evolution of diverse (molecular, cellular, tissue) languages. For these reasons, the processes that initiate a successful pregnancy are, at least at this time, amongst the least understood.

Cell–cell signaling is the current idiom to define ovoreceptivity, which was originally described in chronological terms to explain cooperativity between the embryo and the maternal host (15,18). It is now recognized that these relationships, although conserved, are not identically scheduled in each mammalian species. Modern cell–cell communication language and technology have not explained intra- and interspecies differences. Nor have these intellectual or technological advances helped to resolve the enigma of why the degree of success characteristic of embryo transfer in laboratory rodents (>85%) has not been achieved following embryo transfer in humans, cows, sheep, and pigs (19). While studies have defined time parameters of uterine tolerance for out-of-phase embryos in greater detail (window of implantation), they have not provided much useful information about the developmental status of the uterine endometrium or posttransfer embryos. Periimplantation losses in these species are estimated to be in the range of 40%.

Embryos with no detectable genotype abnormalities may be lost at any time during the periimplantation period because of errors in the communication of development. Either they fail to signal their correct developmental status to the maternal system

or the signal is not received or transduced by the endometrial cells. Years of effort to define reciprocal cell-cell and cell-substrate communication systems have failed to yield practical information that can be used to predictably assure the future of a transferred embryo.

The unexplained early pregnancy wastage in humans and domestic farm animals is a major technical and economic barrier to the success of assisted reproductive technology and the transfer of advances in molecular genetics to species other than the mouse. It is evident that continuing efforts to improve the outcome of these procedures will not be rewarding until the mechanisms regulating successful embryological and endometrial differentiation and their interactions are better understood in each mammalian species.

The uterus is not an idiot savant! Ovoreceptivity, the regulated (endocrine, paracrine) evolution of transient apical uterine epithelium (UE) cell surface adhesivity, the transduction of a trophoctoderm (TE) signal(s) to UE, reception of the TE signals by UE, and translation to the cells of the stromal compartment are functions of the differentiated (receptive) UE cell that are dependent on the integrity of the polarized epithelial cell phenotype (16,17). At some later date, when data become available, the role of reciprocal (uterine stroma) (US)→UE and UE→TE signals will necessarily be integrated into our analysis of these interactions.

Consideration of the relevance of uterine receptivity redirects emphasis from analytical paradigms restricted in scope, i.e., single on/off events as represented by the image of the "window of implantation." More productive, but more difficult, are investigative prototypes that integrate the multiphasic, multifunctional relationships that evolve between TE, UE, and US partners. Pivotal to pregnancy success is that, although each component runs on an individual differentiative clock, and although each responds differently to similar signals and produces a different outcome, each partner exercises only limited autonomy when acting in concert.

Compared with our knowledge of other epithelial cells, our understanding of UE cell biology continues to be rudimentary. During transition from a non-receptive status these postmitotic cells are directed (endocrine, paracrine) to develop a specialized directory of differentiated processes, some repressive, some stimulatory, which are a precondition for the success of the UE cells in their first physical

interactions (secretion, remodeling) with the blastocyst. Specifically the apical surface domain evolves a program of transformation, which integrates a program of signal reception and transduction. These changes abrogate a basic principle of epithelial cell biology and are required to establish receptivity.

Allowing the transient adhesion of two apical epithelial cell surfaces (UE, TE) annuls a fundamental edict of cell biology that disallows apical/apical attachment of two epithelial cells. Abrogation of this law is prerequisite to embryo attachment and the initiation of placentation (16,17). The "immediate" response to this interaction can be accomplished only within this stringently regulated microenvironment. While we have identified endocrine and paracrine factors that model receptivity, the specific biochemical profile, which defines the receptive endometrium, can be described only in the most rudimentary terms. The elements that specifically allow attachment, i.e., establishment of pregnancy, remain unknown.

ROLE OF ADHESION MOLECULES IN BLASTOCYST ATTACHMENT

Recent studies (20) have restimulated interest in analyzing the initial embryo-endometrium interactions as an inflammatory response (21,22). Using principles derived from active research on adhesion molecules (23,24), the modern interpretation of leucocyte-endothelial cell interactions during inflammatory injury provides a productive model for the analysis of embryo-endometrium attachment (20). Studied in terms of differentiating structure and function within defined spatial and temporal parameters, the process of embryo attachment can now be studied in the context of a cascade of inflammatory responses (20,24). The database provided by such analysis prompts a reinterpretation of the concept of ovoreceptivity, which now must be enlarged beyond the temporal constraints of a window of implantation.

Increased understanding of cell adhesion mechanisms has allowed investigation of the molecular events that might underlie the process of embryo attachment and initiation of placentation. In simplistic terms the acquisition of receptivity may be viewed as steroid hormone down-regulation of antiadhesion molecules and/or up-regulation of one or more adhesion molecules at the apical UE cell surface (25). These receptors would interact with cognate

ligands on the outer trophectoderm surface, thereby effecting attachment. Recent attempts to model and analyze the receptive uterus have focused on the biochemical mosaicism of adhesion molecules (integrins, selectins, immunoglobulins, cadherins) expressed on the apical UE cell surface.

Integrins expressed at the cell surface have become the subject of concentrated study because of their relationship with inside/out and outside/in cellular signaling. It is evident from this work (20,24,25–28) that receptivity is not a single receptor/ligand event. Acquisition of a receptive (adhesive) apical UE surface can be described in terms of the differential expression of the individual components of apical surface integrins, i.e., integrin switching, that constitute the apical surface mosaic. However, the specific factors that provoke the aggregation of selected integrins required to activate the signal kinases (focal adhesion kinases) that empower the processes that accomplish embryo–apical UE surface attachment remain unidentified (29). Analysis of the complexity of the receptive apical UE cell surface as posited by the identity and binding affinities of its receptor/ligand complexes would be the least amount of data that could provide insight into the diverse outcomes that may follow the initial attachment event. Spatial distribution and critical concentrations of appropriate receptor–ligand components on the apical UE surface at the time of attachment would signal the expression of a specific downstream program that optimally would direct the development of the fetal–placental unit and result in a live birth.

EXPERIMENTAL MODELS

As a working model it could be proposed that the blastocyst attaches initially to a specific lactosaccharide, LNF-1 (20), an adhesive molecule of moderate affinity. This initial attachment, coupled with sequential interaction with integrin receptors, “A” and “B,” serves to enhance the affinity of the primary binding reaction and stimulate reciprocal blastocyst/UE signaling. Optimally the evoked changes in trophectoderm and UE cell would initiate step 2, which is represented by the activation of different components of the apical surface receptor–ligand program that assures valid TE/UE programmed interactions. Switching, i.e., altering and modulating, the apical surface receptor–ligand interactions (step 1, LNF-1, integrins A and B →

step 2, integrins B, C, and D) redefines the signal components of TE/UE communication in a manner consonant with the progress of normal development. This would be represented as the sequential expression of evolving profiles of integrins and other adhesion molecules, paracrine regulators of migration, and aggregation of kinase activators of intra- and intercellular signaling systems, i.e., step 3, step 4, etc. The progressive differentiation of these events that satisfy the sequence of temporal, spatial, and energy thresholds describes postattachment embryogenesis, fetal development, and the differentiation and maintenance of species placentation that are required to provide the foundation for the successful fetal–placental unit and result in live birth.

Alternately step 1 could be initiated under receptor–ligand conditions, that, although they support the physical attachment of the embryo, initiate an altered step 2, i.e., b, c, and d rather than B, C, and D. Step 2 can progress, but at a suboptimal rate and/or level of activity. The conditions that it regulates support the processes of TE and/or UE differentiation at a level that is dysfunctional but not to the extent that it fails to stimulate step 3, which would terminate pregnancy at this point.

Step 3 could represent, because of the summation of subthreshold temporal and spatial aberrations, dysfunction at an increased, a terminal, level. Thus pregnancy could be interrupted, depending on the initial conditions at attachment, at any number of downstream focal points at which relative developmental deficiencies exceed threshold, i.e., periimplantation and embryonic or fetal losses.

Focusing on embryo–endometrium interactions during the past three decades has produced an extensive library of structural and functional suppositional correlates of uterine receptivity. Categorizing these putative markers whose expression is coincident to the period of ovoreceptivity has served to describe processes operating at many levels of organization and has yielded some new insights (16,17,25,27,28). However, these studies have failed to identify stage-specific markers that actually can be demonstrated to play a principal role in determining the outcome of pregnancy. To correct this deficiency, focus has been directed toward defining the principles of cell–cell communication. These studies of the regulatory biology of embryo–endometrium microenvironment have only just begun to be rigorously analyzed. If the appearance or disappearance of a specific integrin at the

window of implantation actually proved to be more of a correlation than a coincidence, it could be established as a determinant that could be used predictively to resolve the enigma presented by blastocyst attachment and its sequelae (16,25,27,28,30). Specific integrin expression as a correlate of uterine receptivity has yet to be definitely confirmed.

PROSPECTS AND PROJECTIONS

The problems of pregnancy wastage in mammalian populations cannot be simply resolved by an increase in the amount and specificity of research experimentation as practiced in present society. Sociological and cultural practices of the gynecological operating room limit the number and quality of human endometria that qualify for study. This option is even more effectively foreclosed by ethical and legal constraints.

The complexities in organizing and analyzing studies of early pregnancy as noted above have necessarily required that this research become increasingly dependent on knowledge from studies of comparative reproductive biology (31). Resistance to extrapolation of data from farm and laboratory animals for the analysis of human pregnancy wastage, because the data are not perfectly analogous, is diminishing, as it should. Divergence can often prove a better teacher than simulation, particularly in identifying and resolving mechanisms common to the periimplantation phase in all mammals. A number of experimental models focusing on manipulation of gene expression (32), maternal recognition (33), and the development of primary endometrial cell culture systems that respond to environmental signals in a cell-specific manner (16,17) and can be used in the elucidation of reciprocal epithelial-stromal cell paracrine signaling offer the promise of identifying factors that can be proven to be primary determinants of the outcome of embryo-endometrium interactions.

The uterus is not an idiot savant! Rather it provides a complex, multidimensional substrate for trophoblast interaction that integrates the signal languages of different endometrial cellular phenotypes. Thus, observed successful attachment could in fact represent any number of developmental programs. Not every attachment event guarantees a successful outcome to pregnancy. That first, apparently successful, embryo-endometrium attachment

could elicit developmental programs that, proceeding along different temporal and spatial parameters, could in each case produce other, less successful outcomes, i.e., termination of gestation during preembryo, embryo, or fetal development. The primary challenge to reproductive scientists is to find the uterine Rosetta Stone. With this key we could interpret the endocrine-paracrine-regulated changes that define differentiation of individual endometrial cell types in ways that would yield new insight relative to their synchronized development with the developing embryo. This new information could be used predictably to resolve and correct aberrations in embryo-endometrium communication that can now be classified only as infertility.

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