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Is There Any Evidence for Immunologically Mediated or Immunologically Modifiable Early Pregnancy Failure?

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***Purpose*:** Human reproduction is an inefficient process. There is a high rate of loss of early pregnancies, often before the mother (or physician) knows she is pregnant. Genetic abnormalities can explain much of the wastage, but can it explain *all* of the failures? As embryos bear paternal and embryonic antigens foreign to the maternal immune system, could some otherwise normal embryos be "rejected"?
***Methods*:** Critical review of existing data.
***Results and Conclusions*:** Otherwise normal embryos can fail prior to implantation, at implantation, in the periimplantation period as occult/chemical pregnancies, and as clinically evident miscarriages. The maternal immune system and its products (e.g., cytokines) can have innocent bystander effects, and a good case for direct recognition and "rejection" can also be made. The tools needed for accurate clinical diagnosis of such situations require further development and validation. Deliberate modification of the maternal host defence system can improve the chance of success, but the best evidence for efficacy of immunotherapeutic interventions is the situation of recurrent spontaneous abortions, which constitutes only a small percentage of losses. There is also evidence of clinical efficacy for several types of treatment to improve implantation and early pregnancy success.

KEY WORDS: Implantation failure; IVF; occult pregnancy loss; reproductive immunology.

INTRODUCTION

The classic mathematical analysis of reproductive success in England and Wales by Roberts and Lowe 1975 (1) indicated that normal human reproduction is an inefficient process. Only 22.8% of conceptive matings appeared to result in a live birth. In such a study, the "normal" population would include approximately 10% with infertility due to failure to conceive, failure to implant, or a chemical pregnancy (occult loss), 5–11% with a sporadic first trimester miscarriage, and 2–4% with recurrent miscarriages. The fact that only 5–15% of clinically evident pregnancies are seen to fail indicates that most failures of pregnancy are preclinical. Data supporting the estimates of Roberts and Lowe have been presented by others (2). A high rate of loss may be seen as "good," because otherwise the world would be even more overpopulated. Conversely, a high rate of loss is "bad," for those whose desire to have one or more children is thwarted. As specialists trying to help couples with pregnancy loss, we are confronted by our ignorance of the underlying processes causing "physiological" failures in putative "normal" couples, and pathogenic mechanisms in whom we believe there is an underlying pathology meriting intervention.

Roberts and Lowe suggested most pregnancy wastage was caused by "abnormal" embryos (1). From

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the study of spontaneous miscarriage tissue, we know there are significant chromosome abnormalities in 70% of sporadic abortuses, in 50–60% of recurrent abortions where there are no live births, and in 35% of recurrent abortions where there has been at least one live birth (3). Of eight preimplantation oocytes recovered by Hertig *et al.* from otherwise fertile women of age <40, four were morphologically normal (50%) (4). Ignoring the imprecision of small numbers for the moment, these data imply that an additional 27% of otherwise normal embryos are lost at or after the time of implantation. Further, as mating in an outbred human population usually results in formation of an embryo in which 50% of the genetic material (and proteins produced) are potentially foreign to the mother, there is considerable interest in a possible role of maternal immune responses in determining the success or failure of these early pregnancies.

The role of immunological factors and of immunotherapeutic treatments in unexplained recurrent miscarriages has recently been reviewed in detail (5), and the details need not be reiterated here. It is sufficient to say that repeat miscarriage of otherwise normal embryos can be effectively prevented in properly diagnosed cases by applying an appropriate method of modulation of the maternal immunological and inflammatory systems (5,6). The focus of this paper will be preclinical pregnancy failure—what we know about mechanisms and treatment. I will review the data upon which our hypotheses depend, including the results of clinical trials of immunological interventions.

A SYSTEMATIC ANALYSIS OF THE PROBLEM OF ABNORMAL KARYOTYPE

Gametes

Since a high proportion of pregnancy failure can be caused by abnormal numbers of chromosomes in embryonic cells, we must first determine what proportion of oocytes and spermatozoa are abnormal. Combining the data from different studies tabulated by Martin *et al.* (7) and Plachot (8), $906/1927 = 47\%$ of karyotyped unfertilized oocytes were abnormal. The variation among 14 individual studies is large, ranging from 12.8 to 83%, with a mean and standard deviation of $(47.3 \pm 22.3)\%$. A variety of possible explanations could explain such variability. Many of the donors are from infertile relationships, but infertility has a variety of causes, not just karyotype-abnormal eggs. Plachot (8) mentioned that a small unpublished series of 56 oocytes from fertile donors manifest only a 3.6% abnormal karyotype rate, but if that were true for the majority of the "normal" population, it would only strengthen the argument that a large number of otherwise normal embryos are lost. It would be helpful to know if a woman with predominantly normal oocytes or predominantly abnormal oocytes in one sampling period produced a similar pattern in a second sampling. One could then generate a frequency plot per individuals, showing the likelihood in any cycle of there being a normal oocyte. One must also ask if abnormal spermatozoa could make a major contribution to the generation of abnormal embryos that will fail. Martin *et al.* (7) reported from a series of studies that only 10% of spermatozoa were chromosomally abnormal. It is unclear if abnormal spermatozoa could migrate to the site of fertilization, or fertilize a vulnerable oocyte; only 100–200 spermatazoa out of an ejaculate of 40–60 million successfully migrate to the site in the fallopian tube where fertilization takes place (9). The 47% figure for the frequency of abnormal oocytes is remarkably close to the abnormality rate reported by Hertig *et al.* (4). However, for the purpose of this essay, one needs to examine the data on *fertilized* oocytes before accepting 47% as the maximum likelihood estimate for the *normal* population.

Fertilized Oocytes

The next question is what percentage of oocytes fertilze, and of these, what percentage are abnormal and what percentage are normal. Roberts and Lowe assumed a 50% chance of fertilization in situations where timing of sperm exposure was "optimal" (1), but is there any supporting data?

Upon fertilization, the fertilized oocyte releases a factor or factors which, with a contribution from the maternal uterus and immune system cells, leads to generation of Early Pregnancy Factor (EPF) (10). The detection of EPF relies upon a bioassay, the rosette inhibition test, of uncertain biological meaning. The recent demonstration that EPF may be acting on binding to CD2, a known lymphocyte signalling receptor (11), is a helpful antidote to incredulity. There are three studies of serial EPF measurements in otherwise healthy women attempting conception. Smart *et al.* (12) studied 21 cycles in 18 women, and obtained 14 positive results ($14/21 = 67\%$). Rolfe (13) studied 28 cycles in 13 women, and obtained 16 positive results ($18/28 = 64\%$). Fan and Zheng (14) studied a single cycle in 70 women and obtained 35 positive

results (35/70 = 50%). If one pools these data, a fertilization rate of 67/119 = 56% is estimated for conception in a "normal" population. The result is close to the estimate used by Roberts and Lowe (1), and similar to the 48% rate of fertilization in 109 infertile partnership cycles reported by Shahani *et al.* (15) where clomiphene was used to enhance ovulation. From studies of in vitro fertilization, Pieters *et al.* (16) reported 452/752 (60.1%) of oocytes had in fact fertilized, although only 46 required detailed chromosome analysis to show fertilization and these were among a large group of nondividing oocytes defined as IVF "failures" (of which only a proportion could be analyzed).

If the probability of in vivo fertilization in "normals" is 56%, is there any selection whereby normal karyotype embryos are more likely to be fertilized? This information is difficult to obtain. In Pieters' study, 150 IVF failures could be analyzed and 135 could not. Of 127/150 karyotype-normal oocytes, 31% had fertilized, whereas of 23/150 abnormal oocytes, 26% had fertilized. This suggests a 1.2:1 preference for karyotype normal oocytes, but as the result was nondividing, it is difficult to extrapolate. In the study of unfertilized oocytes, 47% were abnormal and 53% were normal. Applying this estimate to the data of Pieters *et al.*, there would be 398 normal oocytes: if 56% fertilized, 23 did not divide and 200 did, then 200/402 (50%) of dividing embryos would have been abnormal and 50% normal. Of dividing embryos, from a variety of studies as summarized in Table I, an average of 63% were karyotypically abnormal. This small difference could be explained by the fact that in my analysis of these studies, the presence of a mixture of normal and abnormal cells (mosaicism) was scored as an abnormal embryo. Where the chromosome abnormality rate in blastocysts was studied, the % abnormal rate was 90%. Munné has related the high percentage of mosaicism to problems with optimizing in vitro culture conditions (25). Therefore, for the present analysis, it seems reasonable to assume that >37% of blastocysts will have a "normal" karyotype, and there may be a slight preference for karyotype-normal oocytes to fertilize. For this analysis, I shall assume an equal chance of fertilization of normal and abnormal embryos, a negligible contribution by abnormal spermatozoa.

Implantation

If we begin with 1000 oocytes of which 47% are abnormal and 56% of oocytes are fertilized, we will have 560 fertilized oocytes of which 267 will be normal and 293 will be abnormal embryos. The % normal blastocysts will be higher than in Table I because of the artifact of culture-induced abnormalities, especially mosaicism.

Implantation has been diagnosed by appearance of the hormone βhCG in maternal serum. There are two studies in which the development of a positive serum β-hCG in a total of 25 EPF-positive women was reported. On average, 48% of EPF-positive women have a positive βhCG (12,14). Continuation to a clinically recognizable pregnancy occurred in only a portion of these patients. On the basis of serial βhCG testing in women attempting conception, a high frequency of implantations which do not lead to clinical pregnancy has been reported. These studies have been summarized in detail elsewhere (9), and may be divided into those with high sampling frequency

Table I. Karyotype Abnormalities in Preimplantation Human Embryos

Investigator	Test	# Oocytes	# Normal	# Mosaic	% Normal
Pre-blastocyst Studies					
Laverge (17) 1987	FISH[a,b]	116	39	22	34
Munne (18) 1993	FISH	30	9	11	30
Delhanty (19) 1997	FISH	93	46	28	49[c]
Noqueiva (20) 2000	FISH[a,b]	14	3	6	22
Wells (21) 2000	FISH	12	3	6	25
Voullare (22) 2000	CGH[d]	12	3	5	25
Blastocyst Studies					
Pellestor (23) 1994	Karyo	118	12	44	10
Sandalinas (24) 2000	FISH	50	6	24	12

[a] FISH: fluorescent in situ hybridization (to identify specific chromosomes).
[b] Intracytoplasmic sperm injection (ICSI) was used in these cases.
[c] Abstract.
[d] CGH: comparative genomic hybridization.

and low stringency for "diagnosis" of a pregnancy where a single low level positive βhCG was sufficient (9), versus high stringency studies where greater certainty was required. Although there is some evidence that the serum βhCG can become positive without physical contact between blastocyst trophoblast and uterine epithelium due to absorption from the lumen or local βhCG production by glandular epithelial cells (26), for the purpose of this analysis, I shall assume a positive βhCG detected using a conventional assay as used in the various studies cited represents physical contact between trophoblast and uterine epithelium. A single positive test is likely to indicate failure at the time of initial contact, and a more convincing set of serially positive βhCG values or higher than threshold values represents a chemical pregnancy which fails to complete nidation in uterine stroma (9). From the studies mentioned above, with low stringency, 45% of implants failed, and with high stringency studies, 17% (9). If we have 269 implanted embryos, 121 will not progress. Of this number 45 would be chemical pregnancies, and 76 would show a transient positive βhCG. Therefore, one may suggest that 76 will fail at or immediately after attachment to uterine epithelium, and 45 of the remaining 194 embryos will fail to survive long enough to lead to a clinically recognized pregnancy. There will be 148 recognized pregnancies. Based upon 560 conceptions, we will have an implantation rate of 269/560 = 48%. The implantation rate from Hertig's in vivo study was 21/36 (=58%) women in the appropriate sampling interval (4), a figure which is quite similar. The clinical pregnancy rate for our 560 conceptions will be 26.6%.

Of the 269 implanted embryos, 128 will be normal and 141 will be karyotype abnormal if each type has the same 48% probability of implantation. How many of each category will be lost between implantation and the time of "diagnosis" of the 148 clinical pregnancies? Let us consider that the frequency of chromosomal abnormalities at birth is sufficiently low (<0.2%) such that even if all of the clinical pregnancies proceeded to birth, the number of karyotype abnormal babies would ≅ 0. Obviously, the karyotype abnormal implants must be eliminated. If the rate of spontaneous abortion of clinically recognized pregnancies is 14–15%, and 70% on average are chromosomally abnormal, then of our 149 clinical pregnancies, 22 miscarry: 126 will be live born, =22.5% of initial conceptions, a figure which is reasonably close to the 22.8% estimate of Roberts and Lowe (1). Of the 22 failures, 15 will be abnormal and 7 normal. To return now to the time of implantation, one can calculate as follows:

	Normal	Abnormal	Total
Number at implantation	128	141	269
Loss at miscarriage	7	15	22
At term	126	0	126
Required occult loss	−5	126	121

Ignoring for the moment the fact that one cannot have a negative occult loss of normal embryos, these calculations suggest all of the losses at implantation, and during the time between when implantation is attempted and diagnosis of a clinical pregnancy is made, will be abnormal embryos. (It is possible some normal embryos could be gained from mosaic embryos in which karyotype-normal cells overgrow the abnormal ones.) One may estimate from Hertig *et al.* (4) that approximately 30% (95% confidence interval 10–50%) of implanted embryos during this period of time will appear grossly abnormal, and so the frequency of chromosomally abnormal embryos might not be as high as suggested by the above calculation. However, not all chromosomally abnormal embryos may appear abnormal. On the other hand, it would be surprising if there was no loss of chromosomally normal embryos during the periimplantation period, but loss at preimplantation and at the postimplantation phase when clinical pregnancies spontaneously abort. It could be true for a normal fertile population that few normal embryos are lost during implantation and nidation: that would still leave open the possibility that in some infertile patients, there was an *abnormal loss* of karyotype-normal embryos during this time period. However, the assumption that a karyotype-abnormal embryo will perform the complex process of attachment and nidation required to complete implantation with the same ease as normal embryos may also be questioned. It seems reasonable to suggest that abnormal preimplantation embryos may have greater difficulty at the implantation phase. A 62% rate of implantation rate of karyotype-normal blastocysts (27) and a 35% rate of implantation of karyotype-abnormal blastocysts would lead to a revised set of calculations as follows:

	Normal	Abnormal	Total
Number at implantation	166	103	269
Loss at miscarriage	7	15	22
At term	126	0	126
Required occult loss	33	88	121

Table II. An Accounting of the Number of Normal and Abnormal Karyotype Losses at Each Stage of "Normal" Early Pregnancy

Stage	Number of normal karyotype	Number of abnormal karyotype	Total number
Ovulation	530	470	1000
If 100% fertilize	477	523	1000
If 56% fertilize	267	293	560
Adjustment if Table I applies	**207**	**353**	
Develop to blastocysts while implantation "window" is still open			
62% implant if N, 35% if ABN	166	103	269
Adjustment if Table I applies	**128**	**141**	
Loss before clinical pregnancy	33	71	−121
Adjustment if Table I applies	**0**[a]	**126**	
Clinical pregnancies	133	15	148
Loss due to miscarriage	7	15	−22
Live births	126	0	126

[a] Infertiles (who do not achieve a clinical pregnancy) would have **133** occult losses.

From this analysis, there may be a significant wastage of otherwise normal embryos in a normal fertile population, more than four times as many losses that occurs via spontaneous abortion, during the luteal phase and menstrual phase of a normal cycle. However, if the frequency of karyotype-normal preimplantation embryos is as low as suggested by Table I, then again all of the losses at implantation and nidation could be loss of karyotype-abnormal embryos *in a normal fertile population*. Since infertile patients will not have 126 term pregnancies, one may infer that this group must loose karyotype-normal embryos before the diagnosis of pregnancy. An accounting of the fate of 1000 oocytes is set out in detail in Table II, and adjustment based on Table I is shown in bold type. Unfortunately, it has been difficult to obtain tissue from failing chemical pregnancies to determine embryonic karyotype. With the development of new ultrasound methods by Coulam *et al.* (28) that can track such pregnancies during the luteal phase, it becomes possible to recover and test a sufficiently large number of failing implantations to provide the data we need using methods such as FISH and immunostaining. Provided >47% of karyotype-normal blastocysts implant, there will be a significant wastage of normal embryos at and shortly after implantation in a normal fertile population, just as there is a large wastage of normal embryos due to failure to implant. *These estimates provide the justification for considering possible immunological mechanisms causing the demise of karyotype normal embryos in couples with normal fertility and consideration of such mechanisms as a possible explanation for increased rates of embryo loss in couples with abnormally low fertility.*

IMMUNOLOGICAL MECHANISMS OF VERY EARLY PREGNANCY FAILURE

When one thinks of the immune system, antigen-specific T effector responses and humoral immune responses (antigen-specific antibodies) are the focus. T helper cells type 1 (Th1) produce Th1 cytokines (e.g., IL-2, TNF-α, IFN-γ, IL-15, IL-18) which promote cellular immunity as seen in allograft rejection, antiviral responses, delayed-type hypersensitivity, and inflammation (IL-1, IL-12, and TNF-α represent additional pro-inflammatory cytokines contributed by macrophages); Th2-type cells produce cytokines (e.g., IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17) which favor antibody responses, and have an anti-inflammatory effect. Of course, antibodies can trigger certain types of inflammation even if Th2 cytokines do not. A Th3-type helper cell releases TGF-β-type molecules that promote tolerance, that is nonresponse (29). This scenario neglects the fact that very powerful innate responses occur, mediated by phagocytic cells that are programmed by primitive recognition mechanisms to distinguish self from nonself (29,30). Indeed, recognition of "danger" signals in the form of pathogen-associated molecular patterns is an important mechanism that activates cytokine production (e.g., IL-1, IL-12, TNF-α, CSF's) by cells of the innate

system (including epithelial cells and macrophages) (30). Indeed, for some cytokines, such as TNF-α, to have their full impact, a danger signal in the form of lipopolysaccharide (LPS) must also be present (31).

Among members of the innate immune system one finds natural killer (NK) cells, and intermediate between the innate and classical antigen-specific immune system one finds NKT cells, and T cells with $\gamma\delta$ receptors rather than $\alpha\beta$ receptors. NKT cells can be $\alpha\beta$ or $\gamma\delta$ (29,32,33). Cells of the innate system do not need to take the time to proliferate and differentiate into effector cells typical of the classical immune system but rather, act immediately; further, they tend to congregate at epithelial surfaces that form the interface between the world of nonself and self, do not usually recirculate via the lymph and blood, do not show long-lived immunological memory, and may have "hard wired" receptors that recognize conserved pathogen-associated molecular patterns usually without the need for presentation of foreign molecules in association with self major histocompatibility complex antigens (MHC) by an antigen-presenting cell (29,30). The relevance of the innate immune system for early pregnancy may be summarized as follows:

(a) As already mentioned, a large number of fertilized normal karyotype oocytes fail during the preimplantation period: either they do not develop to the blastocyst stage when the endometrium is still receptive (i.e. the window of implantation) or they are inhibited from hatching and/or attaching to the endometrial epithelium. Th1 cytokines such as TNF-α can inhibit embryo division, trophoblast attachment, and outgrowth (33). This situation is associated with the clinical condition of endometriosis (34) (and possibly also unexplained infertility where peritoneal fluid contains increase numbers of mononuclear leukocytes). Indeed, any inflammatory response in the female genital tract (e.g. induced by an IUD) can impair preimplantation development and attachment to uterine epithelium (9,35), and increased T cells and decreased NK-lineage cell numbers have been found in the endometrium of women with unexplained infertility and endometriosis (36,37). Fertilized oocytes from endometriosis patients implant poorly, but normal oocytes do not have this problem (38). There are thought to be abnormalities in the endometrium of endometriosis patients (39), but their functional significance for fertilized oocytes obtained from donors without endometriosis is uncertain.

Endotoxin (LPS) is normally present in the environment and serves as a potent "danger" signal to the innate immune system. Stress is also a danger signal. Systemic administration of a low dose of LPS can trigger failure at or shortly after implantation in mice (40), as can stress both in mice (41) and in humans (42). It has recently been suggested that such phenomena represent environmental selective pressure which favors survival of the "best embryos," where "best" means the more genetically diverse (43). It has been suggested that a preimplantation immune response or response at the time of implantation to foreign antigens of the embryos that signal diversity might suffice to activate maternal immunoregulatory cells to dampen or control the inflammatory response to facilitate embryo survival (43,44).

(b) To implant, a brief inflammatory response with a focal infiltration of maternal lymphomyeloid cells such as macrophages is required, and the cytokine IL-1 (which derives in part from macrophages) is a major agonist without which implantation does not occur (at least in IL-1 knockout mice) (45). Colony stimulating factors (e.g., CSF-1 and GM-CSF) which promote macrophage production appear to be required for normal implantation, and Robertson *et al.* have shown using mice that TGF-β in seminal plasma acts in part by stimulating GM-CSF production (46); evidence for human relevance will be reviewed later in this essay. Studies using knockout mice indicate the cytokines IL-11 and LIF are also required (45). The source and interaction of these and other cytokines participating in the events of implantation and nidation have been recently reviewed (45). Patients with infertility and recurrent miscarriages may have defective production of LIF in their genital tracts (47). Transfer of spleen cells from pregnant mice to pseudopregnant mice receiving a transfer of fertilized eggs increases implantation rates, and stimulation of LIF production has been implicated (48). There is no evidence that

systemic stimulation of a woman's immune system by allogeneic leukocytes increases LIF in the genital tract. Local sensitization of one horn of the rodent uterus to allogeneic paternal leukocytes was associated with increased numbers of implantations in that horn (49), but there is no evidence of a similar effect following systemic exposure to allogeneic cells. The data are consistent with the idea that a certain level of regulated inflammation may favor implantation and early nidation, but excessive inflammation and excessive cytokine levels may be harmful. Indeed, recent data from Ledray *et al.* have linked high levels of LIF in uterine wash fluids with infertility (50). In part this may be explained by the existence of different isoforms of LIF with different bioactivities.

(c) Murine blastocysts can be rejected at the early periimplantation stage by classical $\alpha\beta$ T cells reactive against minor (non-HLA-type) paternal antigens, provided the blastocysts are placed at a nonuterine site lacking a mechanism to suppress $\alpha\beta$ T cell activity (51); this mechanism involves production of indoleamine 2,3 dioxygenase (IDO) by a subset of antigen-presenting cells (51–53). Similarly, bypassing such APC by use of the pathogen-associated molecular pattern analogue α GalCer, which activates NK$\alpha\beta$T cells in mice, causes early pregnancy failure corresponding, in timing, to chemical pregnancy loss (51). IDO has been difficult to find once a placenta has formed, and failures at the placental stage of pregnancy are thought to have a quite different mechanism involving activation of coagulation by Th1-cytokine-producing NK$\gamma\delta$T cells; protection via suppression of Th1 responses by IL-10 and TGF-β2-producing $\gamma\delta$T cells has been suggested from studies in mice (51). At this stage of pregnancy, cells with $\alpha\beta$ T cell receptor do *not* react to trophoblast cells whereas T and NKT cells with $\gamma\delta$ T cell receptor do (51). The Th1 cytokine TNF-α is strongly implicated in clinical miscarriages, whereas the same cytokine may be beneficial for implantation and early trophoblast growth (51), depending on its concentration. The occult pregnancy failures triggered via $\alpha\beta$T cells seem to involve deposition of the C3 component of complement as a mediator, rather than TNF-α (51,52). *Only by analysis of biopsies of occult human pregnancy failure will we be able to determine whether similar mechanisms of early pregnancy failure operate in humans.* Blastocysts lack surface expression of classical MHC antigens such as HLA-A, and B (Class I MHC) and HLA-D (Class II) that are associated with recognition of minor paternal antigens by $\alpha\beta$ T cells, and so indirect presentation of such antigens by maternal uterine macrophages is required; αGalCer is presented by the atypical/nonclassical Class 1 MHC molecule CD1 in mice (51), but whether this molecule is expressed on blastocysts or on maternal uterine macrophages in mice is unknown. Atypical Class I MHC molecules such as HLA-G and HLA-E may be present on human extravillous cytotrophoblast, and HLA-G and HLA-E may be expressed by preimplantation embryos (54).

(d) Certain autoantibodies, specifically antiphospholipid antibodies, can impair trophoblast function and have been implicated in causing implantation failure in mice (55). Disturbance of fusion of cytotrophoblast cells after implantation has been suggested as a basis whereby antiphospholipid antibodies may cause early failures in women (56). Indeed, infertility and recurrent miscarriages can be a manifestation of subclinical autoimmune disease (57), and a variety of autoantibodies distinct from antiphospholipid antibodies have been found at increased frequencies in women with pregnancy failures (58,59) Since presence of similar autoantibodies in the normal population has not been linked to a higher risk of pregnancy failure (60), additional factors may need to be present for infertility to occur.

CLINICAL APPLICATION OF IMMUNOMODULATION IN EARLY PREGNANCY FAILURE

There are a several examples of clinical relevance of the limited findings set out above in treatment:

(a) Seminal plasma suppositories, or physiological exposure before embryo transfer in IVF

patients can enhance the rate of implantation (61,62).

(b) Lipiodol, a contrast agent used for hysterosalpingograms, when ingested by macrophages, seems to increase the pregnancy rate based on a recent randomized controlled trial (63).

(c) IVIG (which interferes with autoantibodies and which contains anticytokine antibodies) may improve implantation rates. There is one positive and one negative RCT (64,65). Small sample size, and type of IVIG used may be factors in the outcome. Similarly, heparin + ASA improved success rates in one trial in antiphospholipid antibody-positive patients (66).

(d) Allogeneic leukocytes prevent recurrent miscarriage by activating Th2/3 responses, and the costimulatory molecule OX-2 (CD200) plays a crucial role (5,29,43,51,67). Provided miscarriages are not due to autoantibodies, allogeneic leukocytes appear beneficial to those with primary recurrent abortions (5). Protective effects are lost if the cells are stored overnight before use, even at 4°C (5,29,43,51) Protection occurs almost immediately, and probably by modifying maternal effector cells rather than by inducing an immune response; Mowbray and Underwood showed an alloantibody response (which is against HLA antigens on the donor cells that are not on fetal trophoblast) prolongs protection (5), and this is suspected to represent immunological enhancement of survival of the $CD200^+$ paternal leukocytes. CD200Fc has recently been found to stimulate IDO production (67). However, there is no evidence allogeneic leukocytes enhance fertility in couples with recurrent miscarriages. The pregnancy rate in a meta-analysis of RCT's was 75% in controls and 78.5% in alloimmunized patients (5). In stress-treated A/J mice, allogeneic leukocytes prevent abortions but reduce the number of implantations/rate of pregnancy: this is strain specific (41). If the fertility of some women was adversely effected by leukocyte immunotherapy, the slight increase in pregnancy rate noted above could suggest that some women have their fertility enhanced. The genetic basis for responding one way rather than the other has not been defined, there are no clinical tests that can be used to predict effects on fertility, and mechanisms of positive (or negative) effects of allogeneic leukocytes on early pregnancy remain undefined. The effect of combining allogeneic leukocytes with other modalities has not been tested in a rigorous manner. A relative infertility manifest by reduced family size and increased inter-pregnancy intervals has been associated with sharing of HLA-DR Class II MHC antigens in Hutterite couples (68); the mechanism of this effect is unknown, and there have been no reported studies of immunotherapy in this group.

(e) Anti-pro-inflammatory cytokine drugs such as pentoxyphylline and anti-TNF-α have been suggested to combat infertility, particularly in the setting of endometriosis (34). Further data from randomized trials of adequate size to achieve statistical power (5) is required to assess the value of such strategies.

CONCLUSION

There is tantalizing information suggesting that it may be possible to manipulate the maternal defence system in a way that increases the chance of establishing and maintaining a clinical pregnancy. Systematic application of the tools of science offers the best hope of making progress in diagnosis and effective treatment of patients with failure in early pregnancy. In mediaeval times, much time and effort was expended guestimating how many angels could sit on the head of a pin, and that would have been less of a problem if only there had been some data. Today we do have new methods that make it possible to obtain the data we need, and the future appears promising.

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