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Stress and Recurrent Miscarriage

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Our current understanding into the role of stress in unexplained recurrent miscarriages comes from two different research strategies. The majority of research has examined the role of psychological support within this patient population. This support has been provided in a number of ways ranging from weekly interviews with a psychiatrist or gynaecologist and/or visual re-assurance in the form of ultrasound scans. A comparison of psychological support with an absence of such intervention has found differences in successful pregnancy outcome varying from as great as 84 versus 26%, respectively. It has been assumed that psychological support reduces the miscarriage rate by reducing "stress" within this patient population. In addition it provides indirect support for a role of stress in the aetiology of unexplained recurrent miscarriage. Other studies have attempted to directly assess the effect of personality characteristics on miscarriage rate; these studies have yielded conflicting results.

The mechanism by which stress may be causal in the aetiology of unexplained recurrent miscarriage has not been examined in humans. Animal studies, however, have found that psychological distress can alter immune parameters that may be intricately involved with implantation. These parameters include an elevation of the "abortive" cytokine TNF-α and a reduction in the "anti-abortive" cytokine TGF-β2. Cells that are involved in the release of TNF-α at the feto-maternal interface include T cells, macrophages and mast cells. Mechanisms through which stress may act on these cells are explored and an integrated model is postulated.

Keywords: Recurrent miscarriage; Psychological causes; Stress; Mechanisms; Immunology

INTRODUCTION

Recurrent miscarriage (RMC) is defined as three or more consecutive miscarriages at less than 24 weeks gestation. The observed risk of a woman experiencing a single miscarriage has been conservatively estimated to be around 15% (i.e. 0.15; Regan *et al.*, 1989). The calculated risk of a woman experiencing three

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consecutive miscarriages has thus been estimated to be 0.34% (i.e. $0.15 \times 0.15 \times 0.15$). The observed risk of a woman experiencing three miscarriages is, however, 1% (Speroff *et al.*, 1989). A number of explanations have been provided to account for this discrepancy. These include genetic, infectious, hormonal and immunological factors. However, around 50% of recurrent miscarriages remain "unexplained" (Li, 1998). It is amongst this group of women that factors such as "stress" may play a more major role.

The subjective experience of an event being stressful is dependent on the interplay of a multitude of factors. These factors may be broadly divided into psychological characteristics (e.g. personality traits and coping strategies) and biological factors (e.g. reactivity of the hypothalamic pituitary-adrenal (HPA) axis). There has, however, been very little research that has attempted to directly analyse either of these factors amongst this patient population. Support for a psychosomatic hypothesis comes primarily from research that has indirectly assessed the effect of stress on RMC. These studies have examined the outcome of various "stress-reducing therapies" on miscarriage rate. The magnitude of the stress effect suggested by these studies is significant. However, the quality of the research methodology utilised suffers from a number of major weaknesses making it difficult to extrapolate more broadly.

A further problem with much of the research in this important area of womens" health is the apparent general lack of interest since the 1960s. Consequently, the studies that presently inform us have utilised tools that have become outdated and superseded by better instruments. The absence of analysis with these modern tools further hinders interpretation of the significance of stress in RMC. A number of factors may have contributed to the recent lack of examination of the role of psychological factors. One of these may be a shift of interest towards rapidly developing fields such as immunology. It has, however, become increasingly apparent over the past decade that psychological stress can be "translated" into changes within the immune system *via* the HPA axis (Ader, 1987). Only a few animal studies have specifically examined the relationship between stress and the immune system in the context of miscarriage. However, additional understanding can be gained through studies that have analysed the mechanisms that prevent the "normal" maternal immune system from rejecting the immunologically "foreign" foetus. This paper will explore some of these findings and put forward an integrated model

STRESS AND PSYCHOLOGICAL SUPPORT

A variety of psychologically supportive strategies have been used to examine indirectly the effect of stress reduction amongst RMC patients. Tupper and Weil (1962) examined the effect of "supportive psychotherapy" that involved a weekly interview with a psychiatrist and encouragement to "telephone the psychiatrist at any time". They assessed 38 women, but only 19 were followed throughout their pregnancies. The reason given for the remaining 19 women not receiving treatment was "geographic, etc."; these women then acted as a control group. The study found that the pregnancy survival rate amongst women in the treatment group was significantly higher than the "control" group (cf. Table I). Unfortunately, the method of self-allocation utilised by this study makes extrapolation of the results difficult, as the possibility of a confounding factor cannot be excluded. Tupper and Weil provide further support for their findings by highlighting the fact that the pregnancy survival rate in the control group was comparable to the expected rate of 27% suggested by Malpas (1938). This figure is substantially lower than more recent estimates of a 60% live birth rate amongst women suffering three miscarriages receiving no form of treatment (Speroff *et al.*, 1989).

Stray-Pedersen and Stray-Pedersen (1984) gave "Tender Loving Care" (TLC) treatment to 37 women with previous recurrent miscarriages of unknown aetiology and compared pregnancy outcome with a group of 24 women who received no additional care. The TLC was composed of "psychological support with weekly medical and ultrasound examination". Table I illustrates the

TABLE I Percentages of women with recurrent miscarriage and tender loving care (TLC)

Source	Tupper and Weil (1962) (n)	Stray-Pedersen and Stray-Pedersen (1984) (n)	Liddell *et al.* (1991) (n)	Clifford *et al.* (1997) (n)
TLC	84% (19)	86% (37)	86% (44)	72% (160)
No TLC	26% (19)	33% (24)	33% (9)	44% (41)

significant increase in live births amongst those women in the TLC group. Similar to the previous study, however, the patients self-selected themselves into their allocated group. Although the study claims that women did not differ with respect to age, marital or socio-economic status, the authors' conclusion that "TLC treatment is so effective that it is unethical to withhold it" is questionable, given the methodological limitations.

Liddell *et al.* (1991) assessed the effects of relaxation training on pregnancy outcome amongst 44 patients with unexplained RMC. Patients were recruited after rejection from a larger study investigating the role of immunotherapy in this patient population. The women were seen as early as possible in their pregnancy and referred to a physiotherapy class teaching "relaxation skills". In addition they were asked to use a "relaxation tape" daily. Ten control women were "randomly assigned by clerical staff" to a standard gynaecological clinic from which they were discharged after investigation revealed no underlying cause for their miscarriages. Table I again reveals a striking improvement in pregnancy outcome amongst those women who received relaxation therapy. This study is limited by a number of factors. First, the women recruited for this study had already been rejected from a separate trial in the department. Secondly, the authors assume that allocation by the clerical staff was "random". Finally, the study makes no attempt to explain what the "relaxation" training or "relaxation tapes" consisted of. These factors make interpretation of the results difficult.

Clifford *et al.* (1997) have published the largest and most recent study in this area, in which 201 consecutive women with a history of unexplained RMC were studied. All women were encouraged to attend a dedicated RMC clinic early in their subsequent pregnancies. At this clinic women were seen weekly for ultrasound confirmation of foetal viability and follow up with a gynaecologist. Women who attended the clinic were compared to women who were unable to attend. The results are illustrated above (Table I). No demographic differences were reported between women attending the clinic and those who did not. However, the potential for demographic or other factors to bias the results once again limits this study despite the size of the population examined.

PSYCHOLOGICAL CHARACTERISTICS OF THE RMC PATIENT

As discussed above, the subjective experience of an event being stressful is dependent on the interplay of a multitude of factors. These include psychological and biological factors. Psychological factors include the personality traits of the individual in addition to their coping strategies. Grimm (1962) carried out a study to determine the personality characteristics amongst 61 women who had suffered from unexplained RMC. In addition women were provided with a "brief and supportive type of psychotherapy before and during (a subsequent) pregnancy". A battery of structured psychological tests was administered to patients. This included the Weschler-Bellvue, Rorschach and Thematic Apperception tests. (These questionnaires are rarely used in modern clinical practice as they have been replaced with more sophisticated instruments.) Scores on these questionnaires were compared to a group of 35 women who were either pregnant or had been referred to the hospital with a gynaecological or obstetric problem of "possible

psychosomatic origin". Ten indicators discriminated between the two groups. In summary, RMC patients were found to be more emotionally labile, more emotionally dependent, had greater tension about hostile affect and a greater proneness to feelings of guilt.

The second phase of the study involved re-testing 18 women in the RMC group at 35 weeks gestation in a subsequent pregnancy. A sharp decrease ($p<0.01$) in the "total score" (based on the test indicators) was found amongst women in this group. In addition, the changes found in those women "successfully treated" with psychotherapy were those characteristics that distinguished them from the control group at the outset of the study. The study concluded that a "certain set of personality characteristics typify a sizeable group of abortion-prone women". These characteristics are noted to become less pervasive in those women who go on to have successful pregnancies after psychotherapy. The author does not directly speculate on causality but the reference to "successful" psychotherapy implies that the direction is not coincidental. The study design, however, limits the conclusions that can be drawn. For example, the study would have been more informative if it had also re-tested those women who failed to have successful pregnancies after psychotherapy. In addition, re-testing women at 35 weeks gestation may have been biased by a possibly short-term positive effect of being close to a successful pregnancy outcome as well as by the hormonal milieu at this stage of gestation.

Bergent *et al.* (1997) studied 36 women 6–8 weeks after "at least the second consecutive miscarriage". They were given a different battery of questionnaires aimed at quantitatively measuring anxiety (State-Trait Anxiety Inventory), depression (Beck Depression Inventory), life satisfaction (Life Satisfaction Questionnaire) and any evidence of a somatization disorder (Giessener Beshwerdebogen). The results were compared to a control group of 36 women matched for age and occupation who visited the hospital for a pre-conception check-up. Women in the RMC group were then telephoned 2 years later to enquire about their obstetric history. The comparison between RMC patients and the control group revealed that patients with RMC were significantly more satisfied with their quality of life regarding leisure time, financial situation and occupation. No significant differences were observed in any other variables. Within the RMC group 18 women had a successful pregnancy within 2 years and 18 remained childless. Psychological factors were unable to distinguish between women in either group. The authors concluded that "psychological factors seem to be of subordinate importance as a cause for recurrent spontaneous abortion." Again the study design makes these conclusions premature. One of the major criticisms of the study is that two thirds of the women had experienced two, as opposed to three, miscarriages and were, by definition, not RMC patients. Around half of the "RMC" patients were found to have physical abnormalities that accounted for their condition. As already suggested, women with unexplained RMC may include a subgroup with a higher psychological vulnerability to RMC. If this were true the sample size in this study is likely to be too small to detect it.

This author has carried out a recent study re-assessing these psychological factors using a similar battery of questionnaires amongst a group of 69 women who had suffered from RMC (Craig *et al.*, 2001). The questionnaires included the Beck's Depression Inventory (BDI-II), the Spielberger Trait Anxiety Inventory (STAI-II), the General Health Questionnaire (GHQ) and the St Mary's Miscarriage Questionnaire (SMMQ). This study found significantly elevated levels of depression and anxiety levels amongst this patient population. Although the main weakness of this study was the lack of a control group, it still remains unclear what group of women would provide an appropriate control in this area of study.

THE HYPOTHALAMIC PITUITARY-ADRENAL AXIS

The hypothalamic pituitary-adrenal axis serves as both a "sensor" of psychological stress and a "converter" of this stress into hormone secretion.

These outputs include adrenaline and cortisol, and interest has grown in the mechanisms through which these hormones can be translated into changes within the immune system. This area of study has revealed pathways that may help to explain how stress could be involved in the aetiology of RMC (Fig. 1). To date only animal data are available and extrapolation to the human requires caution.

ANIMAL STUDIES

The Indoleamine 2,3-dioxygenase (IDO) Hypothesis

In mammals the proliferative reaction of the inner lining of the uterus to a pregnancy produces the decidua. This contains maternal lymphomyeloid cells in the epithelium and lymphatic cells, natural killer (NK) cells, B and T cells in the stroma. The immunological paradox of allogenic (i.e. genetically dissimilar) foetal survival in this environment has recently been explored by Munn *et al.* (1998). Their study examined the role of the enzyme indoleamine 2,3-dioxygenase (IDO). This enzyme had previously been found to be expressed by certain macrophages in response to interferon-γ. It is utilised by these cells to inhibit T cell proliferation by rapidly catabolising tryptophan. Human syncytiotrophoblast cells (i.e. the cells lining the placental villi) also express IDO. In addition, systemic tryptophan concentration falls during normal pregnancy. It was therefore hypothesised that IDO expression at the foetal-maternal interface was responsible for preventing immunological rejection of the foetus by the T cells present in the uterine decidua.

To test the hypothesis they mated female mice with syngenic (i.e. genetically identical) males to produce a group of females with syngenic foetuses and mated a separate group of females with allogenic males to produce a group of females carrying allogenic foetuses (the latter group is analogous to the immunological situation in human pregnancies). Pregnant mice in both groups were then exposed to either a pharmacological inhibitor of IDO (1-methyl-tryptophan) or to a placebo. After 9.5 days post conception there were no remaining conceptuses in the pregnant females carrying allogenic foetuses in the group exposed to the inhibitor of IDO. The authors explain this in terms of a failure in the local immunosuppression of T cells. These lymphocytes were then able to recognise the conceptuses as foreign and destroy them. In contrast the mean number of syngenic conceptuses was not affected by exposure to the inhibitor of IDO. This was predicted on the basis that the proliferating maternal T cells would not recognise these conceptuses as being foreign. Conceptuses in those mice who were exposed to placebo were not affected. The hypothesis was supported by a further study that assessed the direct contribution of maternal lymphocytes in the loss of allogenic conceptuses. This involved using female mice carrying a defective *RAG-1* gene *(RAG-1*)*, which prevents lymphocyte development. These mice were then exposed to either the inhibitor of IDO or placebo. It was postulated that in the presence of defective lymphocytes inhibition of IDO would make no difference to the number of conceptuses, which was confirmed by the study. From the IDO hypothesis an increase in miscarriage rate can be expected in pregnant animals fed on a very high tryptophan diet. This is supported by an earlier study by Meir and Wilson (1983), although on a different species. Hamsters fed diets containing normal amounts of tryptophan gave birth to litters averaging either 8.6 (±0.9) or 9.7 (±1.4) young. Additional amounts of tryptophan severely reduced litter sizes by an average of 50%.

The above evidence suggests that IDO may play a pivotal role in foetal rejection and hence miscarriage. Determining the molecular structures that are able to inhibit the activity of this enzyme would be an important next step. With regards to the role of stress in the aetiology of miscarriage the effects of hormones of the HPA axis would be of critical interest. Cortisol has been shown to rise in concentration during mammalian pregnancy (Smith *et al.*, 1990). From an evolutionary perspective it might be predicted that cortisol would either have had a neutral or an inducing effect on IDO. Taylor and Feng (1991) have noted that

IDO is not induced by glucocorticoid and hydrocortisone. However, the effect of adrenaline has not been directly investigated. Interestingly, Noda *et al.* (1983) have examined the effect of adrenaline on tryptophan 2,3-dioxygenase (TDO). This enzyme is involved with tryptophan catabolism in the liver. Their study of primary cultures of rat hepatocytes found that the glucocorticoid dexamethasone (10 μM) and the hormone glucagon (0.5 μM) caused a several fold induction of TDO. However, the addition of adrenaline (10 μM) blocked this effect. TDO is also expressed in mouse decidual cells, indicating a role similar to that of IDO (Tatsumi *et al.*, 2000). Although highly speculative it is possible that adrenaline, secreted in stress, may act to down-regulate TDO, and perhaps also IDO, in the decidua.

Stress, γδ T Cells and Other Immune Parameters

Other studies into the mechanisms through which stress can lead to miscarriage have analysed the role of specific T cell subsets. The majority of T cell subsets have not been investigated as they can only recognise foreign antigens presented to them by major histocompatibility complex (MHC) I and II molecules, and trophoblastic (placental) tissue exhibits a reduction or complete absence of MHC class I and II molecules. T cells bearing γδ receptors have been found to respond to antigen in a MHC-unrestricted manner (Holoshitz *et al.*, 1992; Davis and Chien, 1995). This subset of T cells has recently been isolated within murine (rat/mice) uterine decidua (Van Kaer *et al.*, 1991). Arck *et al.* (1997) therefore investigated the role of γδ T cells during early pregnancy and the effect of their expression in response to stress. Male (*DBA/2J*) and female (*CBA/J*) mice were mated. A random selection of some of the pregnant female mice were then subjected to an ultrasonic sound stress from a battery-powered rodent repellent device for a single 24 h period on day 5.5 of pregnancy. The paradigm was then repeated after injecting 100 μg of anti-γδ T cell receptor (GL4) i.p. on day 5.5 (i.e. the same day as the sound stressor). The study found that the miscarriage rate (% resorptions) in the mice not injected with GL4, increased from 8.5% in the non-stressed group to 36.4% in those mice subjected to the sound stressor. However, after injection of GL4 the resorption rate in the mice subjected to the sound stressor was 12.7% lower than expected ($p<0.05$). Thus injection of anti-γδ T cell receptor (GL4) led to a significant decrease in stress-triggered resorption.

The authors hypothesise that this may be attributable to γδ T cells producing the abortive cytokine tumour necrosis factor-α (TNF-α). This is supported by the finding that levels of TNF-α in decidual γδ T cells decreased from 6.2% in the control group to 1.1% in the GL4 injected mice. Previous research by this group (Arck *et al.*, 1995) revealed an increase in the "abortive" cytokine TNF-α and a reduction in the "anti-abortive" cytokine transforming growth factor-β2 (TGF-β2) in response to stress. Although the mechanism by which TGF-β2 may protect against resorption is unclear, a number of mechanisms through which TNF-α may cause resorption have been demonstrated. This includes inhibition of DNA synthesis and proliferation in trophoblastic cells (Hunt, 1989) in addition to the possible activation of NK cells into lymphokine-activated killer (LAK)-type cells able to damage foetal trophoblast cells (Chaouat *et al.*, 1990). Arck and her colleagues point out that stress induced TNF-α production can be derived from other immune cells in the uterus, including macrophages and mast cells, which may account for GL4 only partially preventing stress triggered resorption. In addition they speculate that some γδ T cells may produce pregnancy-protective cytokines such as TGF-β2.

This group has postulated a separate mechanism through which stress may lead to an elevation in TNF-α from macrophages and mast cells which hinges on the neurotransmitter substance P. Previous studies revealed that substance P containing nerve fibres innervate the reproductive organs of mammals (Taurig *et al.*, 1984). In addition, *in vitro* studies have revealed that substance P induces the secretion of cytokines including TNF-α (Ansel *et al.*, 1993). Since mast cells and macrophages are known to release TNF-α and may also possess substance P receptors the group carried out a study to determine the role of substance

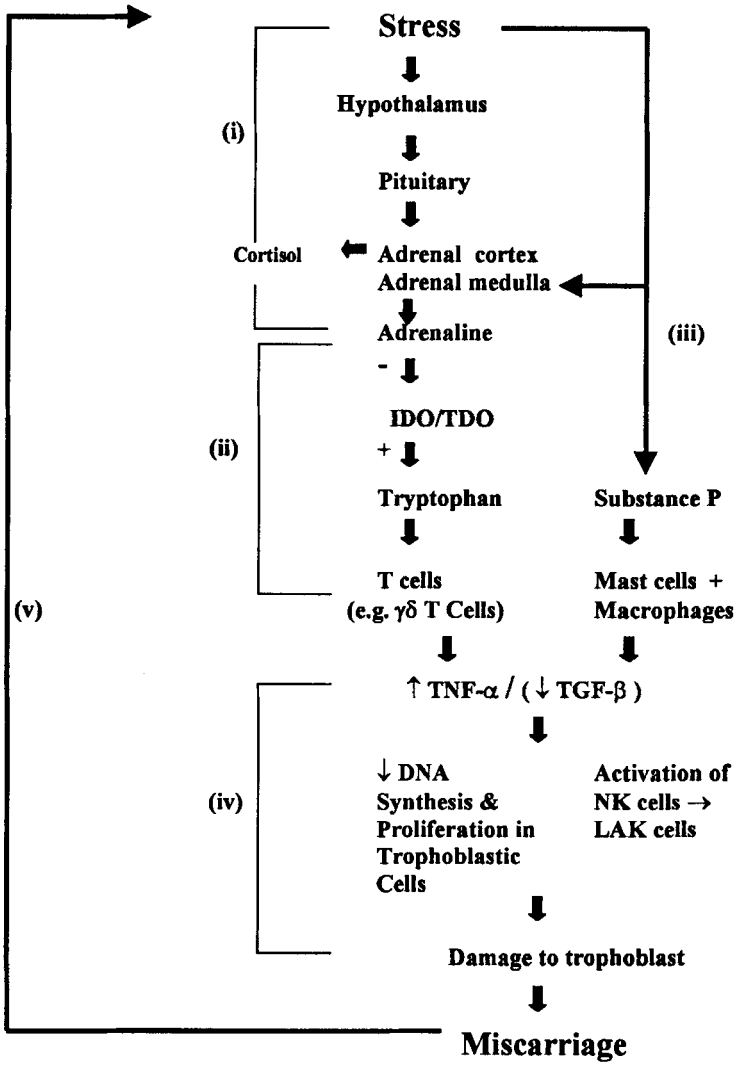

FIGURE 1 Possible mechanisms of miscarriage induced by stress. (i) Pituitary and adrenal stress responses, including secretion of adrenaline. Responsiveness to stress may be dysregulated, and women with RMC experience more anxiety, with over-stimulation of the secretion of adrenaline. Supportive care during the early stages of pregnancy might act to reduce the over-responsiveness of this axis. (ii) Illustrates how adrenaline might down-regulate expression of the enzyme IDO. This may in turn serve to increase local levels of tryptophan at the foetal–maternal interface. The elevated tryptophan levels could then act to allow proliferation of maternal T cells at this interface. The majority of T cells would be unable to react against trophoblastic tissue due to the down-regulation of MHC class I and II molecules. However, specific T cell subsets, such as those bearing γδ receptors, may play an important role, and perhaps be up-regulated by stress. This up-regulation may not usually be apparent in a tryptophan deplete environment; inhibition of IDO may unmask the destructive potential of specific T cells exemplified by this subset. (iii) Stress may lead to release of the abortive cytokine TNF-α *via* substance P. (iv) Two mechanisms through which trophoblastic tissue may be damaged by TNF-α. (v) Subsequent miscarriages may increase stress and depressive symptomatology possibly leading to further dysregulation of the HPA axis.

P in stress-triggered resorptions. The study (Arck *et al.*, 1995) involved depleting substance P containing neurons in mice by injection of the neurotoxin capsaicin at birth. When these females were later mated and subjected to stress, the expected stress-triggered increase in miscarriage did not occur. Furthermore, the increased TNF-α levels observed in the non-treated mice was not observed in those treated

with capsaicin. Further support for the hypothesis was provided by injecting a substance P receptor antagonist into a group of non-capsaicin-treated pregnant mice. As predicted by their hypothesis stress did not lead to a significant rise in TNF-α or miscarriage rate in this group.

The main problem, however, with each of the studies described above is the degree to which they may be extrapolated into furthering understanding into RMC in human pregnancy. Arck *et al.*, for example, used a strain of female mice that were known to "miscarry" in response to sound stress, which has not been found with other strains of mice.

AN INTEGRATED MODEL

A model that attempts to integrate information generated from animal and human studies is illustrated in Fig. 1.

CONCLUSION

The most persuasive studies in support of the hypothesis that stress plays a major role in women suffering from RMC examine the effects of supportive care amongst this patient population. However, these studies have two major shortcomings. First, they failed to assess stress prospectively before and after treatment in a controlled manner. It remains unclear whether these methods of supportive care (TLC) are reducing stress or acting through another undefined route. Secondly, studies to date have failed to randomise women to treatment or control groups, which means that a selection bias confounds the results. A prospective, randomised controlled trial is needed to assess the role of supportive care amongst women suffering from RMC. Ideally any such study should use both biological and psychological means of measuring stress at different points in a longitudinal analysis.

Animal studies have provided some insight into how psychological stress may be converted into physiological rejection of the developing foetus. Two possible mechanisms have been examined in this review. Here I have proposed an integrated model that allows the generation of testable hypotheses. It is apparent, however, that this important area of womens' health is in need of further study. Modern techniques offer the potential to answer many of the questions posed by previous research and a multi-disciplinary approach is required to answer them.

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