Recurrent Pregnancy Loss: Towards More Accurate Diagnosis and Treatment

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In order for pregnancy to develop from conception to delivery there are two main prerequisites: the embryo must be normal and capable of further development, and the maternal environment must be conducive to further development. However, in current clinical practice little attempt is made to determine whether pregnancy loss is of maternal or fetal origin. A presumptive diagnosis is usually made after investigations for maternal causes only. If one result of an investigation is abnormal, it is assumed that the cause has been diagnosed and treatment is offered. This approach may be misleading and counterproductive since it precludes the possibility that the embryo may be abnormal and incompatible with life. There are two known embryonic causes of fetal demise – structural malformations and chromosomal aberrations.

Fetal causes of pregnancy loss

Chromosomal aberrations account for 50–60% of sporadic miscarriages. Three studies have assessed chromosomal aberrations in recurrent miscarriage. Both Ogesawara et al. [1] and Stern et al. [2] reported that 50–60% of abortuses are chromosomally abnormal in women with two or more miscarriages (mean number of miscarriages 3.8 and 3.5 respectively), and Carp et al. [3] reported an incidence of 29% in women with three or more miscarriages (mean 4.7). Ogesawara et al. [1] demonstrated that as the number of miscarriages increases, the incidence of chromosomal aberrations decreases. Fetal karyotyping, although officially recommended as an essential part of the investigation of recurrent pregnancy loss by the Royal College of Obstetricians and Gynaecologists [4] in the UK, is not routinely investigated in Israel or most other centers in the world. Therefore, if treatment is offered for a presumed maternal cause of pregnancy loss – such as uterine septum, antiphospholipid syndrome, thrombophilia, or alloimmunity – up to 60% of subsequent pregnancies may be lost due to chromosomal aberrations. Furthermore, when the results of treatment for presumed maternal factors are assessed, they will be skewed. If the figures are not corrected for chromosomal anomalies, efficacious treatment will be interpreted as ineffective.

Fetal structural anomalies, which are not chromosomally based, may also cause errors in accurate diagnosis. Seventy percent of miscarriages are blighted ova [5], in which embryonic demise occurs so early in pregnancy that ultrasound can only visualize an empty pregnancy sac. In these cases, it is impossible to tell whether any rudimentary embryo may have been structurally abnormal. Another 20% of pregnancy losses are missed abortions with fetal death in the first trimester; however, as the resolution of ultrasound improves it may be possible to diagnose structural anomalies earlier in pregnancy. Some missed abortions have enlarged yolk sacs [6] that may represent a fetal anomaly. It is conceivable that structural anomalies may be caused by extrinsic maternal factors. The serum of habitually aborting women is toxic to pre-implantation embryos and blastocysts [7]. Ten day mouse embryos have a higher incidence of malformations, such as exencephaly, microcephaly, anophthalmia, cardiac defects and yolk sac anomalies, if cultured in the presence of habitually aborting women's serum [8], hence there is a high rate of subsequent pregnancy loss. The incidence of anomalies could be reduced in some of these embryos if immunoglobulin G interchange is first performed on the habitually aborting women's serum [9]. It has been shown that the effect of two teratogens – cyclophosphamide and quinoxalinedimethanol – can be lessened in mice embryos by the intrauterine injection of allogeneic paternal splenocytes [10] or the TH-2 cytokine granulocyte macrophage colony-stimulating factor [11] This 'immunization' caused a twofold decrease in the number of resorbtion sites and a threefold decrease in the number of anomalies such as exencephaly, micrognathia and microphthalmia. The manipulation of anomalies has not been studied in humans, but it is possible that some women with recurrent miscarriage are forming abnormal embryos, and some of these patients may be amenable to therapy for maternal factors.

Clinical presentation

It is usually assumed that all pregnancy losses – from biochemical pregnancies up to fetal deaths or premature labor at 20 or 28 weeks – constitute a single homogeneous entity. However, different causes of recurrent pregnancy loss may have different clinical presentations, for example cervical incompe-

tence causes a clinical picture of second-trimester painless dilatation and abortion of a live fetus. Chromosome 16 trisomy presents as missed abortion of a 2 mm embryo [12] and triploidy as a blighted ovum [12]. The typical presentation of antiphospholipid antibody syndrome is growth retardation probably leading to fetal death in the second or third trimester [13]. Hence these antibodies have been reported to occur in approximately 10% of unselected patients with recurrent pregnancy loss [14] but in 30% [15] of patients with recurrent second-trimester pregnancy loss. Activated protein C resistance has been described as causing fetal death mainly after 20 weeks [16], or more significantly after 28 weeks [17,18]. The septate uterus has also been described to cause expulsion of the live fetus in the second or third trimester, after "mini-labor" [19]. Alloimmune pregnancy loss probably presents as primary (in which all the pregnancies are lost) or tertiary abortion (miscarriages, followed by a live birth, then further miscarriages) of blighted ova or early missed abortions. If a study assesses treatment in all patients with a laboratory feature, e.g., APLA, the beneficial results that may be obtained for treatment of a specific clinical presentation will be obscured by the failure of treatment in inappropriate patients.

Assessment of results

There is a third confounding factor: the prognosis varies according to the following features, each of which increases the chance of another miscarriage. These include advanced maternal age, a higher number of previous miscarriages, primary as opposed to secondary abortion (live birth followed by a string of miscarriages), concurrent infertility [20], and a previous eukaryotypic abortion [1,5,21]. The number of previous abortions is the most important predictive factor. If a trial compares patients with five abortions in the treatment arm and three abortions in the control arm, as in certain past investigations, the trial will most probably show no treatment benefit.

Ideally, any form of therapy should be assessed on a randomized basis, and should preferably be double blind. The treatment and control groups should be matched for the above predictive factors and appropriate clinical presentation. The results should then be corrected to exclude fetal chromosomal aberrations in the subsequent pregnancy. No trial of treatment for recurrent pregnancy loss has used these criteria; instead they assessed treatment in all patients with three or more abortions. Randomized trials have been conducted to assess paternal leucocyte immunization, immunoglobulin, progesterone and human chorionic gonadotropin supplements. No placebo control trials have been undertaken to assess treatment for antiphospholipid syndrome, hereditary thrombophilias, or surgery for uterine anomalies. Below are some examples of trials whose failure to show beneficial effects may have been due to their failure to perform fetal karyotyping, assess a specific clinical presentation of pregnancy loss, or take predictive factors into account.

Although still highly controversial, many authorities consider the immune system to have a major role in the surveillance of pregnancy, and that the mother needs to mount an appropriate immune response, possibly involving the release of TH-2 cytokines that prevent the activation of natural killer cells. An inappropriate response leads to the release of TH-1 cytokines, activation of NK cells, and abortion. Numerous publications have reported increased numbers and activity of NK cells in recurrently aborting women [22,23]. Immunopotentiation (either active with paternal or third party leucocytes, or passive with intravenous immunoglobulin) has been used to modulate cytokine release and NK activation [24–26], thereby preventing further pregnancy losses. Despite the use of paternal leukocyte immunization for over 15 years, double-blind trials have not produced a consensus as to efficacy. In order to settle the controversy, a meta-analysis was performed on the 449 patients from eight double-blind randomized trials [20]. This meta-analysis concluded that there was a statistically significant beneficial effect of approximately 10%. Other important conclusions became apparent in the analysis: namely, treatment was effective in the primary but not secondary aborter, in the patient who produced anti-paternal antibodies as a result of immunization, and that the efficacy increases with the number of abortions. When the results were corrected for predictive factors the benefit was 20%, but no correction was made for fetal chromosomal aberrations or for the gestational age of the pregnancy when lost. There have been two subsequent trends to widen and to narrow the indications for treatment. Ober et al. [27] carried out a double-blind trial using the broadest inclusion criteria. They included patients with prior pregnancy losses up to 28 weeks even if the loss was due to premature labor (C. Ober, personal communication). Moreover, secondary aborters in whom the losses were not necessarily consecutive chromosomally aberrant fetuses were not excluded from the trial but were considered failures of treatment, as were patients who did not conceive within 12 months of treatment (even if they had previous infertility). Immunizations were stored in the fridge overnight, reducing efficacy. The trial concluded (not surprisingly) that immunization is ineffective in preventing miscarriage. Our team [28] attempted to assess paternal leukocyte immunization in a more homogeneous group with five or more first-trimester abortions (and therefore a poor prognosis); the higher number of miscarriages the lower the likelihood of chromosomal aberrations [1]. Primary and tertiary (but not secondary) aborters had a statistically significant 27% higher live birth rate if immunized. The odds ratio for a live birth was 3.99 times higher (confidence interval 2.61–6.10). Six randomized control trials [29] assessed passive immunization with intravenous immunoglobulin. Four claim that there is no

APLA = antiphospholipid antibodies

NK = natural killer

benefit whereas two show a significant benefit. Our group [29] has used immunoglobulin in women with five or more abortions. In these circumstances, the advantage in terms of live birth rate after treatment was 20% ($P < 0.05$).

Progesterone is an essential prerequisite for pregnancy to develop. The progesterone receptor antagonist mifepristone is widely used both in Israel and abroad in order to induce artificial abortion. It has long been assumed that a defective corpus luteum may produce inadequate progesterone, leading to failure of implantation and placentation [30]. Summarizing the literature on progesterone support in recurrent miscarriage, Daya [31] noted three papers from the 1950s and 1960s that claimed no beneficial effect from progesterone. However, a pooling of the results in a meta-analysis [31] showed a statistically significant benefit. Since then, the tendency has been to administer progesterone empirically, while making no correction for chromosomally abnormal pregnancies; thus the results are almost impossible to analyze. Low progesterone levels might be a result of abortion rather than the cause. In the blighted ovum or missed abortion, there is no villous circulation after embryonic death. A trophoblast with no villous circulation may produce insufficient hCG [32] to allow the corpus luteum to secrete progesterone. Until now no attempt has been made to determine whether low progesterone levels are a cause of abortion or a consequence of embryonic demise due to another mechanism such as chromosomal aberration. The results of hCG supplementation are also inconclusive. Three trials of hCG supplementation in recurrent pregnancy loss in the 1970s and 1980s showed hCG supplements to be beneficial in sustaining pregnancy. Harrison et al. [33] performed a double-blind study in 1992 and reported that hCG had no beneficial effect. Since then hCG supplementation has almost dropped into disuse. However, as with progesterone, if the results of all the studies on hCG supplementation are pooled, there is a 20% benefit (137 pregnancies terminating in live births in 156 treated patients compared to 80 of 121 untreated patients). The only trial that assessed a more homogeneous group of patients is that of Quenby and Farquarson [34], who reported that hCG supplementation improved the subsequent pregnancy outcome in oligomenorrheic women, but not in normomenorrheic women. Double-blind trials in which both progesterone and hCG are tested using the criteria listed above are sorely needed.

The most recently described maternal factors to cause pregnancy loss are the hereditary thrombophilias. These are genetic tendencies to thrombosis. As thrombosis in decidual blood vessels is one of the mechanisms whereby APLA can lead to pregnancy loss, the hereditary thrombophilias have become implicated as causes of pregnancy loss. As with the other causes of pregnancy loss described above, it has been claimed that thrombophilias can cause pregnancy loss at all stages of pregnancy [35], while some researchers have doubted whether thrombophilias ever cause pregnancy loss. As stated above, various authors [16–18] are tending to show that hereditary thrombophilias cause late rather than early pregnancy losses. Anticoagulants such as heparin or low molecular weight heparins (enoxaprin or daltaparin) may be an appropriate treatment. A multicenter study is currently underway to assess the dose of anticoagulant necessary to prevent further pregnancy loss in patients with thrombophilias and recurrent pregnancy loss. However, if sub-analyses are not performed for different clinical types of pregnancy loss, or corrected for chromosomal aberrations, the effective population that can be helped (patients with normal emryos and late pregnancy losses) may have their results obscured by the larger number of patients who cannot be helped.

Antiphospholipid antibodies, even if passively transferred, were described many years ago as a cause of pregnancy loss [36]. It is generally accepted that APLA should be treated in recurrent pregnancy loss, but the beneficial effect of treatment has never been shown in a placebo-controlled trial. Comparative trials comparing one form of treatment to another have been performed, e.g., heparin and aspirin to prednisone and aspirin [37], and heparin and aspirin to aspirin alone [38]. Carp et al. [39] reported that treatment does not reduce the incidence of recurrent first-trimester abortion, and Lockshin [13] has described the clinical picture of growth retardation and second- or third-trimester fetal death. A placebo-controlled trial is awaited.

Uterine anomalies have also long been recognized as causes of recurrent pregnancy loss. Since the introduction of hysteroscopic surgery for the resection of uterine septae, fibroids or adhesions, laparotomy with its associated morbidity has been largely discontinued in Israel. Again, reports describe the benefit in terms of anatomical correction or compared to the patient's history [40]. However, since the presence of a septum or fibroid does not prevent fetal chromosomal aberrations, a trial using the above criteria is necessary.

Conclusions

It seems that some embryos are inherently abnormal. These include the chromosomal anomalies, which account for a higher proportion of losses in patients with few pregnancy losses. They are not amenable to any form of therapy. Others embryos have been made abnormal by maternal factors involving cytokines and other immune mechanisms. They may be amenable to treatment. Other embryos may be normal but lost due to maternal factors. They should respond to treatment. Clark and Daya [41] have shown that if 30% of a habitually aborting population have fetuses with chromosomal aberrations it will be relatively easy to see a beneficial effect of treatment for maternal factors, whereas if 70% have chromosomal aberrations there will be no such beneficial effect. While the criterion of three or more pregnancy losses up to 20 or even 28 weeks is used for inclusion in a double-blind randomized trial, it is unlikely that any mode of treatment will be found to be beneficial.

The clinician therefore has a difficult problem. There are

hCG = human chorionic gonadotropin

many protocols dictating how to investigate recurrent miscarriage. However, finding a cause of miscarriage does not mean that the cause has been diagnosed. In the case of two or possibly even three miscarriages the prognosis is good. No treatment has been shown to improve the live birth rate, and reassurance and tender loving care may be the only treatment required, as suggested by Quenby and Farquarson [42]. In patients with a higher number of miscarriages and a poor prognosis, the only treatment to have been investigated is immunotherapy. In light of the results of fetal karyotyping, it may be necessary to reassess all the other modes of treatment. Double-blind randomized trials will then need to be performed in patients with a specific condition, and the results corrected for confounding factors.

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