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Chapter

Abortions in First Trimester Pregnancy, Management, Treatment

Panagiotis Tsikouras, Theodora Deftereou, Xanthoula Anthoulaki, Anastasia Bothou, Anna Chalkidou, Fotini Gaitatzi, Ioannis Tsirkas, Arsou Chalil Bourazan, Eirini Bampageorgaka, George Stanulov, Elefterios Chatzimichael, Spyridon Michalopoulos, Panagiotis Petsidis, Georgios Iatrakis, Stefanos Zervoudis, Maria Lambropoulou, Werner Rath and Georgios Galazios

Abstract

The miscarriages' investigation should include a familiar history, gynecological examination and a full laboratory testing including hormonal control, as well as karyotype, maternal immune control and thrombophilia testing. If the physician suspects the cause of abortions is chromosomal due to heredity, a special blood test (karyotype) for the pair is recommended. Chromosomal abnormalities are the most common reason for first trimester abortions, and are impossible to be prevented. Based on the above data, abortion and the subsequent possible infertility should not be considered as a personal failure for the woman and the treating physician. Nowadays, medical advancement provides many options combined with psychological support can actually reduce the miscarriages' risk.

Keywords: spontaneous abortions, recurrent abortions, diagnosis, therapy

1. Introduction

Spontaneous abortions are considered as one of the most common complications in pregnancy. Abortion is defined as the ejection of the fetus until the 20th week and is clinically classified based on ultrasound evaluation. Threatened abortion is usually accompanied by vaginal bleeding without affecting the pregnancy. Incomplete miscarriage is described as incomplete passage of conception products. Complete, when all pregnancy products are spontaneously aborted. Septic abortion when endometrial infection coexists. Finally, recurrent abortions that are defined when three consecutive abortions occur before 20th weeks of gestation [1–5].

More than 80% of miscarriages occur up to 12 weeks. The percentage of clinically recognized abortions (appeared after 6th week) is estimated to be 12–15%. More analytically, 2 miscarriages occurred in 5% of women and ≥3 miscarriages in 1–3% of women [1–5]. Recurrent miscarriages consist of a serious problem for women with physical and psychological consequences. Abortions and especially recurrent miscarriages are considered as heterogeneous groups in regard to etiology [1–5]. Two or more causes can coexist, whereas about 50% of cases are characterized as idiopathic as a result of genetic, anatomical, endocrine, genetic, anatomical, endocrine, autoimmune factors or infections exclusion [1–5].

The number of clinically recognized miscarriages to total number of pregnancies is about 10–15%, while the equivalent of early abortions based only on human chorionic gonadotropin (hCG) measurements is actually much higher (50–60%) and interestingly before and after the implantation it is 30% but are not perceived [1, 6–10].

A positive correlation has been identified between the mother's biological age and the incidence rate of spontaneous abortions. So, a progressive increase of 10-fold afterwards the age of 40 is observed compared to younger women aged <35 years [1, 6–10].

Regarding the gestational age in first trimester and the abortion risk, this counts to 4 and 2%, respectively, in 6th and 8th week of gestation [1, 6–10]. The risk of recurrence in the next pregnancy is minor except of cases diagnosed with congenital uterine abnormalities [1, 6–10].

1.1 Recurrent miscarriages

Recurrent miscarriages are defined as three or more consecutive embryos losses weighing under 500 g. According to bibliography we can find different definitions for miscarriages [1, 11, 12]: two or more miscarriages of clinical pregnancies [7]; three or more miscarriages of the first trimester or one or more miscarriages of the second trimester; three or more miscarriages <14 weeks [1, 11, 12]. Frequency appearance is of quite large (1 in every 300 pregnancies).

Recurrent miscarriages (frequency 1–3% among couples of reproductive age) usually occur during the first trimester and relative risk increases with the number of previous miscarriages [1, 11, 12]. Consequently, after the first miscarriage, this risk reaches 24%, after the second one to 26% and after the third it amounts to 32% [1, 11, 12]. About 10–15% of all clinical recognized pregnancies are aborted and the theoretical risk for three consecutive pregnancy losses is 0.34% [1, 11, 12].

Receiving history includes:

- the gestational age of spontaneous abortions
- the certification in the presence of embryonic pole and heart function
- the symptoms related to the antiphospholipid syndrome and family history of automatic abortions

The possibility to predict the risk of recurrence depends on several factors like as maternal age, fetal parental karyotypes gestational age, presence of various maternal laboratory findings [1, 11, 12].

About 80% of abortions occur during the first trimester, 50–60% of them are based on genetic abnormalities. The risk of pregnancy loss is 2–5% after the recognition of heart function, 5% for women who report two miscarriages and finally 1% of women mentioning more than three miscarriages [1, 11, 12].

Investigation of recurrent pregnancy loss (RPL) begins with personal history, followed by laboratory, genetic, hormonal, anatomic, immunologic, thrombophilic factors and infective reasons that can affect pregnancy outcome. It is quite often that 2 or more factors coexist [11–20]. In half of cases, etiology cannot be identified, so it is described as "Recurrent miscarriages of unknown etiology" [1, 11, 16].

2. Clinical examination

During the clinical examination, a gynecological examination and a check for hyperandrogenemia and hyperprolactinemia signs should be done [1, 17].

3. Laboratory check

A laboratory control includes:

- Karyotype in couple
- Hysteroscopy or hysterosalpingography (HSG)
- Anticardiolipins and LA
- Control of progesterone levels in the middle of the luteal phase cycle
- Check the levels of FSH,LH,PRL and testosterone (2–5th day of period)
- TVS (transvaginal ultrasound) [1, 13–20].

It is of great importance in thrombophilia examinations control to include V Leiden factor and prothrombin 20210 mutation [1, 15–20].

4. Anatomical factors

Anatomical abnormalities that can cause miscarriages are typically recognized using hydrosonohysterography (HSG), hysteroscopy, laparoscopy, possible in same cases magnetic resonance and recently three-dimensional ultrasonography.

Congenital anomalies malformations of the female reproductive tract uterine anatomical abnormalities, such as bicornuate uterus or uterine diaphragm, polyps, heart-shaped ultrasound fibroids are results from failure completion of bilateral duct elongation, fusion, septal resorption of müllerian ducts [13–15].

Especially with regard to fibroids, they may block the development of early pregnancy, but their influence on spontaneous abortions is also affected by other factors such as age and hormonal disorders. Müllerian anomalies occurred in 8–10% of women, who had three or more consecutive spontaneous abortions. The fibromuscular tissue in septate uterus is poorly vascularized fibromuscular tissue that is associated with the highest pregnancy loss rate in some studies reporting an average of 65%. Except the above-mentioned abnormality, high frequency for pregnancy loss is noticed in didelphys, bicornuate and unicornuate uterus [13–15].

Intrauterine adhesions (Asherman syndrome) are acquired uterine defect resulting from infection, endometritis and unsuccessful curettage, which is associated with recurrent miscarriage, oligomenorrhea and amenorrhea and bad prognosis.

Recommended treatment in these cases includes balloon catheter, administration of estrogen and progestin medication [1, 16–18].

Uterine cavity abnormalities including submucosal, intramural >40 myomas, polyps are associated to poorly vascularization of endometrium led to failure of implantation, placenta tissue development and contribute to pregnancy loss. Cervical insufficiency is described as an acquired uterine anomaly, which is depending in painless cervical dilatation, effacement and inability of the uterine cervix to retain the amniosac is the commonest reason for abortion in the second trimester [16–18]. Exposure of the embryo in diethylstilbestrol DES and a variety of environmental factors like thalidomide, infectious agents and ionizing radiation affects the uterine morphology by triggering changes in both the location and amount of HOXA/Hoxa expression in the development of Müllerian ducts. No prospective studies exist. The HOXA genes along the Müllerian ducts, influencing the development of Müllerian ducts are: HOXA9 Oviduct, HOXA10, HOXA11 Uterus, HOXA11, HOXA13 Cervix and HOXA13 Upper vagina [1, 19–21].

5. Chromosomal abnormalities

Genetics reasons of recurrent pregnancy loss be subdivided in embryo abnormalities resulting of known parental genetic pathology and embryo aneuploidy in parents to be chromosomally normal A variety of genetic factors including aneuploidy (gain or loss of a chromosomal), chromosomal imbalance resulting from harbored translocations, inversions, deletions, duplications within chromosomes, single gene mutations led to recurrent pregnancy loss (RPL) [1, 22–33]. In 3–5% of couples with RPL, the ratio of parental chromosome abnormalities in contrast to the general population is 0.7%. The most common chromosomal abnormalities leading to RPL are balanced translocations. The first chromosomally abnormal abortion was reported in 1961. Chromosomal abnormality is approximately responsible for half of the clinically diagnosed abortions in the first trimester. About 50% of them are autosomal trisomy, 20% monosomy XO, 20% polyploidy and 10% variety of other abnormalities [33–35].

5.1 Karyotype examinations in aborted fetus

- Karyotype in couple
- No need for molecular karyotype
- No need for microdeletions control

About 25% of cases exist in the first trimester, in which although the embryos are normal and euploid, they cannot develop properly. The reasons include women with müllerian agenesis and other significant anatomic abnormalities. In the second trimester, the abortion incidence due to a chromosomal abnormality is <20%. Structural chromosome abnormalities (Robertson-type balancing and translocations).

X-inactivation chromosome (?)

aneuploidy of spermatozoa (?).

Recurrent abortion (RA) as well as repeated IVF failure (RIF) has a common underlying factor which is the significant increase in the rate of chromosomal abnormality [35]. The results also suggest that in women with recurrent abortions,

the transfer of normal embryos improves the pregnancy rate and live-birth rate in both younger and older women. In these cases, preimplantation genetic diagnosis is recommended, testing for structural chromosomal aberrations like translocations, inversions, removing cells from the resultant embryo or oocyte evaluating the cells for genetic abnormalities and determine the optimal embryos for uterine transfer [35–37]. Last year, novel technologies like microarrays, fluorescence in situ hybridization and biopsy of embryo blastocyst are widely used [34–36].

5.2 Lifestyle and environment

Women with RPL have reproductive difficulties because they are concerned about various toxins and agents within the environment. It is of great importance to counseling these couples in health care institutions to have current and accurate information and to avoid exposures to these substances [37, 38]. The rate of spontaneous abortion is positively associated to cigarette smoking, alcohol consumption, obesity, body mass index (BMI, weight in kilograms divided by square of height in meters) >30 kg/m^2, caffeine intake (excess of 300 mg/day) and ionizing radiation [37, 38].

6. Immunological factors

In some case, a failure to activate a normal control mechanism to prevent an immune reaction against self is observed and this subsequently led to autoimmune response. Natural killer cells are attached to the cytotrophoblast of the embryo. However, the mechanism by which such cells may or may not affect the embryo is not proven. When implantation occurs, there is a slight inflammatory response. Patient with infertility and recurrent miscarriages develop less prominent reaction that may prevent the embryo from implanting [39, 40]. Autoimmune abortions are thought to be caused by the presence of autoantibodies that already exist in the woman against membrane phospholipids, thyroid antigens, nuclear antigens, syncytiotrophoblast cells or against other organelles or tissues [39, 40].

About 10–15% of all women have antinuclear antibodies regardless of medical history of RPL. In cases of presence of antinuclear antibodies, the possibility of successful pregnancy outcome is independent of antibodies existence [39, 40].

Autoimmune factors causing RPL are: antiphospholipid antibody syndrome, aPL antibodies (anticardiolipin antibodies and lupus anticoagulant), ß2 glycoprotein antibodies, phosphatidyl serine. Antibody hemeostasis in systemic circulation is different between men and women, just because women need to be better equipped, so that they can cope with the required immune tolerance in fetal antigens derived from them [39, 40].

The “thermostat” is therefore positioned higher in women, as a result, autoimmune diseases have much higher incidence in women. If autoantibodies are found in relation to a pathological condition, this means: whether they are the pathogenetic factors of the disease (autoimmune hemolytic anemia); whether it is the result of a previous pathological process (autoantibodies against cardiac muscle after its destruction) and whether they are causative agents without damaging themselves, which is the most common, as is believed [40, 41].

If the antibodies are present in the mother’s serum as a result of allogeneic stimulation during pregnancy, then they are not considered to be autoantibodies unless they exist previously [40, 41]. Until now, there is no common point of

the effect of pregnancy on the production of autoantibodies. Although no clear increase in autoantibodies has been found during normal pregnancy, an increase in some of them has been reported in pathological pregnancies, most of which have been described as antiphospholipid antibodies [40, 41]. In the group of recurrent miscarriages, 15% were certified positive findings for the lupus anticoagulant or antiphospholipid antibodies or both of them. It is important to notice that lupus anticoagulant is not synonymous with systemic lupus erythematosus (SLE), where it is found in only 5–15% [40, 41].

6.1 Antiphospholipid antibodies

Antiphospholipid antibodies (APAs) are a family of immunoglobulins which react with anions of phospholipids or anions of phospholipid-protein complexes in the cell membrane of the syncytiotrophoblast [1, 41, 42].

The finding of aPL antibodies is associated to adverse pregnancy outcomes such inducing vessel thrombosis of the surrounding placental maternal unit, placenta infarction, and fetal death. The primary mechanism in the first trimester depends on a deleterious effect directly on trophoblastic cells, inhibition of secretion of human placental chorionic gonadotropin, and the expression of trophoblast cell adhesion molecules (a1, a5 integrins, E, VE-cadherins). The most widely used are anti-cardiolipin (diphosphatidyl-glycerol) [1, 41, 42].

Others are anti-phosphatidylserine anti-phosphatidylethanolamine, anti-phosphatidylcholine, anti-phosphatidylinositol, and phosphatidic acid. Large variation in APHA measurements between laboratories and in the same laboratory for the same patient and great fluctuation in the values during pregnancy are observed.

It is questionable whether the same or other substnces (anti-β2-glycoprotein I) have similar impact according to recurrent pregnancy loss [42–45].

Possible action of APAs on miscarriages:

Abnormalities in endothelial cell function of vessels (decrease in production of arachidonic acid prostacyclin and a relative increase of thromboxane which is a potent vasoconstrictor and promotes platelet aggregation).

Obstructive angiopathy (reaction with anion phospholipids exposed after vessel damage).

Platelet stimulation and/or adhesion (in damaged platelets, APAs bind phosphatidylserine to the structural element of the inner membrane of the platelets and promote platelet aggregation and thrombus formation).

Placental infarction (microscopic arterial thrombosis and necrotic fibrous deposition have been found). It also appears to be due to reduced flow in the vessels, as has been found to occur in the umbilical and maternal arteries in patients with lupus or APS. This situation resembles a destruction of the vessels through antibodies after heart transplantation, coronary bypass, or angioplasty [42–45].

Inhibition of protein C stimulation in S. These two proteins, after their activation, inactivate clotting factors Va and VIIa. Stimulating their stimulation creates an increased tendency for coagulation.

Reduction of levels of annexin V, a protein with potent antithrombotic effect on the surface between trophoblast and endothelial cells.

Effect on placental function: (ACA inhibits the secretion of gonadotropin secretion from placenta, which can act on the secretion of hormones from the placenta, negatively affecting the viability of the fetus).

Phospholipids bind to the surface of trophoblast, and this results in direct destruction of cells, inhibition of syncytia formation, decrease of hCG production and defective penetration into maternal peristalsis.

6.2 Autoimmune factors

Antiphospholipid syndrome: high levels of antiphospholipid antibodies and history of miscarriages and/or endometrial death and/or thrombosis—risk of autoimmune in subsequent pregnancy ~90% [46, 47].

6.3 Clinical criteria

Vascular thrombosis (one or more clinical episodes of venous, arterial, or small vessel thrombosis in any tissue or organ).

Gestational complications (one or more recurrent miscarriages after the 10th week of gestation, one or more preterm births and one or more recurrent miscarriages before the 10th week of gestation).

Laboratory criteria: cardiolipin antibodies (IgG or IgM anti-cardiolipins, at moderate or high levels in two or more measurements over a period of at least 6 weeks between them).

Lupus anticoagulant (in two or more measurements at least 6 weeks apart) [44–47].

7. Antiphospholipid syndrome: management

Steroids (complications: pregnancy and prematurity) are not recommend based on current publication evidence. It is reported that the maternal and fetal complications increase without affecting the pregnancy outcome and live births [43–47].

7.1 Prednisolone, between 10 and 20 mg daily dosage

It may prevent recycling in the circulation of cardiolipins or suspend the discharge of embryo toxic factors or factors associated with HLA. In addition, it lowers NK (CD56+/CD16+) cell percentage. It has been associated with pregnancy hypertension, diabetes mellitus, and mainly with premature labor and low-weight new-born babies. Aspirin should be given preconceptually. **Aspirin in low dosage** (80–100 mg daily) may suspend cyclooxygenase (COX) action on platelets, by suspending the composition of thromboxane thrombosis and thus preventing vascular thrombosis in placental blood vessels. At discontinuance after around 32 weeks, heparin (does not pass the placenta) should be started after the first positive pregnancy test and should be continued until of labor to avoid thrombosis risk: hypo-heparin, for example, **heparin of low molecular weight,** one injection daily. Anticoagulant action (reinforces the action of antithrombin III), while it may bind AFAs, thus prevents chorionic villus sampling (CVS) phospholipids from being destroyed, by assisting in the successful implantation in the early stages of pregnancy [43–47]. Thrombocytopenia and osteoporosis check-up. Discontinuation after 34 weeks of pregnancy and prior to giving birth. **(Now in labor)**.

It appears that combining aspirin and heparin has the best results. Patients should start taking heparin as early as possible when pregnant and continue until labor and during puerperium [42–50]. Combination of aspirin and heparin is associated with better results. Heparin subcutaneously, for example, low molecular weight heparin one injection per day may prevent recycling of circulating anti-cardiolipins or suppress the secretion of embryotoxic agents or HLA-related agents. It also reduces the percentage of NK (CD56+/CD16+) cells [46–50]. It has been associated with gestational hypertension and diabetes mellitus and mainly with premature labor and low birth weight neonates. Combination of aspirin with heparin, aspirin

with prednisolone, or all three is associated to satisfactory results. It seems that the combination of aspirin and heparin works best. Heparin should be started as soon as possible in pregnancy and should be maintained until the labor and postpartum especially when the risk of thrombosis is high [42–50]. Intravenous immunoglobulin therapy IVIG (no superior to the combination of aspirin and heparin) Intravenous injection of high doses of gamma globulin (300–500 mg/kg body weight). An increase inT-immunosuppressive cells, decreases the activity of natural killer cells, inhibition of transport by the mother's placenta of IgG, inhibition of Fc receptors in macrophages and, especially, multivalent immunosuppression. To avoid the adverse reactions of heparin therapy, it is recommended to add calcium 600 mg twice daily and vitamin D supplementation 400 IU daily to decrease the osteoporosis risk. The platelet count should be weekly examined in the first two weeks after treatment with heparin because bleeding could occur due to heparin induced thrombocytopenia [42–50].

8. Endocrine factors

Mentioned here are polycystic ovary syndrome (PCOS) due to high levels of luteinizing hormone (LH), corpus luteum (CL) deficiency, nonregulated diabetes mellitus during conception period, thyroid malfunction, thrombophilic factors, alloimmunological factors: PCOS, menstrual complications, hypertrichosis, polycystic ovaries, and resistance to insulin. The contribution of endocrinological factor as reasons of RPL including luteal phase deficiency, untreated hypothyroidism, abnormal glucose metabolism hyperprolactinemia, and diminished ovarian reserve is average by 8–12% [51–54].

8.1 Corpus luteum deficiency

Corpus luteum malfunction association with RPLs still remains a hypothesis, despite the fact that there are studies that reveal that it is responsible for 12–28% of cases. Luteal phase deficiency is defined as an inability of the corpus luteum to secrete progesterone either in increased satisfactory amounts or for too short duration. This inability to function is established by alteration of preovulatory estrogen stimulation, which led to poor oocyte quality and a poorly functioning corpus luteum. The diagnosis should be confirmed either with endometrial biopsy which is not recommended as diagnostic modality or if serum progesterone levels are <10 ng/ml [51–54]. Strategy of treatments of corpus luteum malfunction has a wide variation and includes administration of progesterone or human chorionic gonadotropin induction or a combination of these. Progesterone administration either as intravaginal suppositories 50–100 mg or as intramuscular injections 50 mg IM is considered necessary only within RCTs [51–54].

8.2 Hyperprolactinemia

Hyperprolactinemia is an endocrinopathy which led to infertility and abortions due to anovulation. It is not clear whether it is associated with RPLs. Increased prolactin levels interact with the hypothalamic pituitary ovarian axis, reducing the folliculogenesis or leading to a small duration of luteal phase. Studies reveal that it affects progesterone discharge at luteal stage; however, this situation has not been confirmed in humans. A randomized control trial including 64 hyperprolactinemic women with RPL treated with bromocriptine was associated with a higher rate of successful pregnancy, whereas PRL levels were significantly higher in women that miscarried (85.7 vs. 52.4%) [55–58].

8.3 Dopamine agonists

Currently, there is no sufficient evidence for effectiveness of dopamine agonist evaluation in preventing future miscarriage in women with idiopathic hyperprolactinemia and a history of recurrent miscarriage [55–57].

8.4 Diabetes mellitus

Women with nonregulated DM I: diabetes mellitus (DM) in women with RPLs is associated with a higher incidence of spontaneous abortions in relation to women with euglycemic metabolism preconceptually. A well controlled diabetes mellitus decreases the rates of recurrent pregnancy loss. Testing for fasting insulin and glucose and hemoglobin A1c usually have an increased modality for the evaluation of insulin resistance. The metformin administration seems to improve pregnancy outcome and is it safe in the first trimester [55–57].

8.5 Thyroid gland disorders

It is well known that hypothyroidism without therapy increases the risk of abortion. Treatment before attempting a pregnancy is clearly recommend as well as keeping a TSH level between 1.0–2.5 UIU/ml in the first trimester. In cases with TSH levels higher than 2.5 MIU/ml, levothyroxine should be started at a minimum dose of 50 μg/d.

Anti-thyroid Abs is associated with RPLs when detected before the start of pregnancy or at an early stage.

Hypothyroidism is involved with obstetric complications like infertility, abortions, anemia, preeclampsia, placental abruption, fetal death, preterm birth, and low birth weight [55–58].

8.6 Alloimmune dysfunction

- Elevated CD56+ lymphocyte levels
- Increased NK cell levels in the secretory phase of endometrium
- Higher levels in NK cells during the endometrium secretory phase. Strong association between maternal type Th2-cell immunity and successful pregnancy outcome. Recurrent abortions: associated with immunity type Th1 (lF-γ, TNF, lL-12, 58)

8.7 Diminished ovarian reserve

Increased levels of FSH in the early follicular phase of menstrual cycle are significant for diminished ovarian reserve. In the least years, another marker antimüllerian hormone is better to identify the number of follicular units for recruitment. It is recommended that women with RPL visit healthcare services to have appropriate counselling to treat endocrinological disorders [59].

8.8 PCOS

Polycystic ovary syndrome (PCOS) is associated with increased frequency of RPL and has an uncertain prevalence, because factors associated with PCOS such as obesity, insulin resistance, LH rise, and hyperandrogenemia may be the

reason and not PCOS as a whole [60–68]. The incidence of abortions in spontaneous ovulation is difficult to determine. Diagnostic criteria for this heterogeneous disorder have not been present in the past. Hypersecretion of LH and elevate androgen levels possibly led to RPL [60–68]. The association of excess androgens and RPL is not clear. The hyperinsulinemia in PCOS that is a consequence of insulin resistance involving plasminogen activator inhibitor-1(PAI-1) which inhibits plasminogen activation and subsequent fibrinolysis, has potential thromboembolic effect that makes women with PCOS in high risk for recurrent pregnancy loss [60–68].

8.9 Why LH rise may result in abortion

- premature oocyte maturation
- hyperandrogenemia may impact on oocytes
- high androgens may affect endometrium

8.10 Role of androgen abortion

- androgens may affect endometrium
- in vitro studies show inhibitory effect of androstenedione
- in endometrial cell growth and activity (glycodelin secretion)
- glycodelin inhibits endometrial immune response to the embryo [60–68]

8.11 Endometrium

- gene hoxa10 is thought to be essential for implantation.
- the expression of this gene is decreased when testosterone is elevated.

8.12 Hyperinsulinemia: abortion

Preimplantation environment is affected by decreasing:

1. expression of glycodelin—which inhibits endometrial immune response to the embryo and
2. IGF—binding protein-1, which facilitates adhesion progress at the fetomaternal interface. Plasma plasminogen activator inhibitor-1 concentrations are increased in hyperinsulinemia leading to hypofibrinolytic state and thrombophilia [60–68]

8.13 PCO—abortion: thrombophilia

Plasminogen activator inhibitor gene (PAI-1) activity (i.e., hypofibrinolysis) is elevated in PCO.

Metformin reduces gene activity from 42.5 to 12.4 U/ml, that is, correct tendency for thrombosis which improves uteroplacental flow.

9. Metformin treatment

PAI-1 activity fell 44% in women with live births.
PAI-1 activity increased 19% in women with abortion.

10. Pregnancy loss and possible beneficial effects of metformin

1. Decreased levels of androgen may improve endometrial function; IR resistance is decreased

2. Glycodelin and IGF-1 protein expression is corrected

3. Glycodelin inhibits endometrial immune response to the embryo

4. IGF-1 improves the adhesion process at the fetomaternal interface

5. Hypofibrinolytic activity is reduced by decreased PAI-1 activity (which induces hypofibrinolytic activity) [60–68].

11. The prevalence of abortion in PCO is probably elevated

1. Obesity very probably implicated

2. Insulin resistance probably but may INDIRECTLY affect the parameters which are influenced by metformin

3. The role of LH needs to be elucidated

4. Endometrial factor probable

5. Oocyte factor probable

6. PAI-1 factor probable

7. Further prospective studies needed to elucidate the significance of these factors

Usually, women with PCOS require different treatments due to variety of reasons so we can use metformin, diet and infertility drugs. Therapeutic management combines normalization of weight and administration of metformin to reduce mainly the RPL rate [60–68].

12. Infections

Any infection with high fever can lead to miscarriage rubella virus, cytomegalovirus passing the placenta and affecting the fetus, malaria, mycoplasma, and trypanosomiasis.

Several studies have confirmed the role of infections as a cause of miscarriage especially in the second trimester of pregnancy; however, their role in the first trimester miscarriages remains unclear [69–74].

12.1 Infective factors' mechanisms of action

Toxic metabolic bio-products, endotoxins, exotoxins, or cytokines may have a direct effect on the uterus and the fetoplacental unit chronic endometrial infection following after lineal infection (M. hominis, Chlamydia, Ureaplasma urealyticum, and HSV) may affect the fetus implantation. Fetal infections are possible to cause fetal death or severe malformations incompatible with fetal livability (rubella, parvovirus B19, CMV, HSV, and syphilis). Placental infection probably causes placental deficiency with consequent fetal death. Amnionitis in the first trimester may have a similar effect on chorioamnionitis in the third trimester (causing premature labor). Various microorganisms with such effect, as L. monocytogenes, are suspected [69–74].

12.2 Correlation between the various supposed mechanisms of automatic abortions and specific infectious factors

Mechanisms:

1. Embryotoxicity

2. Placental deficiency

3. Endometritis/endocervicitis

4. Amnionitis

Microorganisms: rubella, parvovirus B19, CMV, HSV, syphilis, Chlamydia, Mycoplasma, Ureaplasma, and various Gram-positive or Gram-negative bacteria (*L. monocytogenes*).

None of the above-mentioned infectious agents are usually confirmed to lead to RPL. Each high fever infection may lead to pregnancy loss. Viruses such as rubella and cytomegalovirus infection (CMV) go through the placenta and affect the embryo, as well as lead to malaria, chlamydia, mycoplasma, and trypanosomiasis [69–74].

Quite a few studies have confirmed that infections are to blame when it comes to miscarriages, especially during the second trimester of pregnancy; however, their role in the first trimester miscarriages remains unspecified.

12.3 Recurrent miscarriages of unknown etiology

New techniques in molecular biology and genetics could recognize the importance of "locality" for mutations. Detection of new mutations in immunological and other molecules is involved in the pathophysiology of abortions. It is of great importance to immediately start appropriate antibiotic therapy based on a test of cure culture, when cervical and vaginal infections are identified and to extend the treatment for both parents [75, 76].

12.4 Thrombophilia

Pregnancy is a condition that predisposes to hypercoagulation. The pregnant woman is in a state of increased tendency for coagulation (hypercoagulable state). The action of the fibrinolytic system decreases during pregnancy,

particularly in the placenta, mainly due to an increase in inhibitors of the plasminogen activator [77–84].

Pregnancy and coagulation mechanisms include the following:

- Increase of coagulation precursors **(procoagulant factors)**
- Decrease in levels of physiologically existing anticoagulant factors (**naturally occurring anticoagulants**)
- Reduction of fibrinogenolysis
- "Modified" maternal response to hemostasis (**'disordered' maternal hemostatic response)** [77–84]

Correlation not quite clear!

Pregnancy is a state of hypercoagulation (hypercoagulable state). This hypercoagulable state do not necessarily causes thrombosis and the miscarriage is not due to thrombosis.and a lot of patients with miscarriages. This predisposition for thrombosis may lead to malfunction in the fetoplacental unit. A disorder in the balance between activators and inhibitors of plasminogen can lead to defective placentation [77–84]. The infiltration of trophoblast in arcuate arteries is essential for implantation, placentation, and consequently a regular continuation of pregnancy. Defective penetration is a common pathological finding in placenta preparations by women with excretion and also preeclampsia or intrauterine fetal growth delay [77–84].

Placenta abnormalities include excessive implantation and placenta accreta.

Increasing thrombophilic factors and five more frequent thrombophilic polymorphisms: a) V Leiden factor; b) MHTHFRC 677T; c) MTHFRA1298C; d) Factor VA1299H; and e) factor II G20210A are predisposition to venous thromboembolism (VTE). None of the five thrombophilic mutations, alone or in combination, was found to significantly increase the risk of miscarriages [77–84].

12.5 Thrombophilic factors

V Leiden: women with RPLs and V Leiden mutation: there is no discrimination test for those who will have recurrent miscarriages from those that have a term pregnancy.

Treatment: prophylactic administration of heparin without the confirmation of RCT is prescribed for known mutations of the factor V Leiden and also for the cases that there is indication of placental thrombosis [77–84].

C677T MTHFR polymorphism: previous studies have shown conflicting results between the MTHFR C677T genotype and the recurrent miscarriages.

5,10-methylentetrahydrofolate reductase catalyzes the conversion of 5,10-methylentetrahydrofolic acid to 5-methyltetrahydrofolic acid.

5-methyltetrahydrofolic acid takes part in the methylation of homocysteine in methionine.

Substitution of a cytosine molecule by a thymine molecule at position 677 increases the incidence of homocysteine and thrombophilia.

The reduced activity of MTHFR and hyperhomocysteinemia is clinically manifested when lack of folic acid coexists [77–84].

Treatment: administration of 0.5–2 mg of folic acid leads to homocysteine normal levels [77–84].

12.6 Combination of multiple mild thrombophilic factors

12.6.1 Plasminogen activator inhibitor (PAI)

Increased activity of PAI: genetic factors, metabolic disorders of insulin resistance syndrome, hypertension, smoking, etc.

PAI-1: the major physiological inhibitor of plasminogen activation plays a central role in fibrinolysis.

PAI-2: trophoblast and macrophages. Increased PAI activity has been linked to recurrent miscarriages.

4G polymorphism of PAI-1 gene is associated with high levels of PAI-1 and reduced fibrinolytic activity.

Homozygous for 4G has been complicated with preeclampsia, prematurity, IUGR, and placental ablation.

12.7 Combining multiple subtle thrombophilic factors

12.7.1 Factor XII deficiency (Hagemann)

- low molecular weight heparin (LMWH)
- heparin
- LMWH vs. heparin
- one injection per day
- anti-Xa follow-up is not necessary
- decreased risk of osteoporosis
- reduced risk of thrombocytopenia
- safe for the fetus as it does not pass the placenta
- more likely outcome in obstetric complications [77–84]

12.8 Increase of homocysteine and thrombophilia

As mentioned before, decreased MTHFR activity and hyperhomocysteinemia are only obvious as long as there is folic acid deficiency. Substitution with folic acid prevents any phenotypic expression of C677T polymorphism.

Prediction for women with polymorphism who are going to have a spontaneous abortion [77–84]:

Treatment: the administration of 0.5–2 mg folic acid reduces homocysteine levels to normal [77–84].

Homocysteine: homocysteine is an amino acid formed as an intermediate of metabolism of methionine. Elevated blood levels of homocysteine (hyperhomocysteinemia) consist an important cardiovascular risk factor, which in recent years have significance similar to hypercholesterolemia [85–90].

The detection of women with congenital hyperthyroidism that have much more frequent complications in gestation has led to an investigation of the association between homocysteine and pregnancy complications associated with placental

vascular lesions such as placental abruption, preeclampsia, abortions, fetal death, and restriction of intrauterine fetal growth. Hyperhomocysteinemia is also associated with spinal tube deficits due to folic acid insufficiency. The result that women with hyperhomocysteinemia of relative cause show complications much more frequently has led to the investigation of obstetric complications with homocysteine, which are relevant with vascular placental failure such as placental abruption, pre-eclampsia, recurrent miscarriages, stillbirth, and intrauterine growth restriction [85–90].

Methionine consist of an important amino-acid that participates in cell growth and division by providing methyl groups in the biosynthesis of t-RNA, DNA, and proteins. Methionine constitutes a necessary amino acid that plays a crucial role in cellular increase and division by providing methyl groups to t-RNA, DNA, and protein biosynthesis. Homocysteine is synthesized after a methyl group transposition from methionine. The 50% could be catalyzed with a sulfureted group transposition into cystathionine. The remaining 50% of homocysteine may be reformed to methionine by removing a methyl group from two sources: (1) the metabolism of tetrahydrofolic acid (THF) and (2) the catabolism of betaine. Three enzymes take part in these metabolic pathways: methionine synthetase (MS), cysteine synthetase (CBS), and methyl-tetrahydrofolic acid reductase (MTHFR). Vitamin B6 (pyridoxine) consists of a coenzyme in the CBS and MTHFR function, while B12 is a coenzyme in the MS function. The adequacy of folic acid is necessary for both functions. The vascular endothelium may only produce CBS and MTHFR, rendering it more sensitive to disturbances in homocysteine metabolism [85–90].

Homocysteine levels >15–16 μmol/l in nonpregnant women and >6–8 μmol/l in the third trimester of pregnancy are considered abnormal. In pregnancy, homocysteine levels decrease progressively and reach a minimum in the second trimester, while increasing slightly in the third trimester. Causes of fluctuations are estrogen, blood dilution, and increased metabolism of homocysteine in the liver, as well as its removal to the fetus. Mild disturbances in homocysteine metabolism can be detected if its levels are measured 6 hours after methionine administration. In this case, levels >51 μmol/l are considered pathologically out of pregnancy, but for pregnancy, there are no measurements [85–90].

Generally, the increase in basal levels of homocysteine represents deficits in remethylation, whereas the increase in homocysteine after methionine loading reveals deficiencies related to the transfer of the sulfur group.

Hyperhomocysteinemia may be related to mutations in genes controlling the production of CBS and MHTFR and environmental factors such as B6, B12, and folic acid deficiency. Drugs that interfere with the metabolism or absorption of these vitamins, decreased homocysteine excretion in chronic renal failure, and other causes (hypothyroidism, hepatic failure, malignant anemia, and cancer) cause hypercholesterolemia [85–90].

It is worth highlighting cases with genetic mutations of the enzymes that cause hyperomyeloidemia, because they are common and appear to play a role in the course of pregnancy [85–90].

12.9 Laboratory criteria

Antibodies vs. cardiolipins: anti-cardiolipins lgG or lgM, in medium or high levels in 2 or more cases, with a time distance of at least 6 weeks between them.

Lupusanticoagulant: in two or more cases with a time distance of at least weeks between them.

- Antiphospholipid antibodies

- Acute infections
- Medicine (chlorpromazine and hydralazine)
- Chronic infections (syphilis and hepatitis C)
- No thrombotic effects
- Antiphospholipid syndrome
- Recurrent abortions
- Endometrial death
- Pre-eclampsia
- IUGR
- B2 glycoprotein 1
- Annexin-V: a protein with strong, anticoagulant action.
- Multiple placental micro-thrombosis [85–90].

13. Pregnancy and hyperhomocysteinemia

The existence of high homocysteine levels in the blood has a harmful effect on the placenta and decidua and is associated with the appearance of recurrent abortion and placental abruption. Hyperhomocysteinemia has been found to cause more complications such as pre-eclampsia, stillbirth, and deceleration of intrauterine growth [85–90].

13.1 Hyperhomocysteinemia and spinal tube deficiency

Hyperhomocysteinemia and spinal tube deficiency (NTD) are associated with insufficient MS and MFTHR function, leading to homocysteine accumulation. Reduced methionine methylation and methyl group (necessary for myelin creation) deficiency are responsible for the above complications and not homocysteine effects [10]. Low folic acid levels or reduced intake in cases with increased need (heat sensitive MTHFR) are responsible for spinal tube deficiencies, but also homocysteine level increase. Hyperhomocysteinemia has also been associated with congenital abnormalities on the face and body. Administering 500 mg folic acid for 4 weeks prior to conception or even at the first stage of pregnancy has been found to reduce homocysteine levels by 22% [90–95].

The gene C677T and MTHFR frequency combined with the dietary habits of a population are the reason for variety in NTD appearance in different populations. In Holland, homozygous C677T of MTHFR polymorphism carriers is at 10–16% NTD risk compared to 5% of witnesses. A1298C, a second type of polymorphism, was discovered with just as high risk NTD levels in case of homozygosity [90–95].

13.2 Hyperhomocysteinemia and pre-eclampsia

Several researchers compared patient groups with witnesses, found an increased hyperhomocysteinemia frequency, as well as increased C677T of MTHFR mutation

frequency in women with pre-eclampsia. So, they were led to the conclusion that it may also constitute a genetic factor in pre-eclampsia manifestation [90–95].

In large woman study groups where homocysteine levels were evaluated in the second trimester of gestation either prospectively or recursively, it was found that the relevant pre-eclampsia risk in women with hyperhomocysteinemia was between 1.32 and 3.2%, while in primigravida, it reached 9.7% and in those who were obese, it reached 6.9%. Certainly, patient choice (with heavy, premature pre-eclampsia) has a lot to do with the various levels of pre-eclampsia in incidence of appearance of hyperhomocysteinemia in the bibliography. In a prospective study of 1049 pregnant women, at their 16th week of gestation, homocysteine levels did not appear to be different amongst patients with an uncomplicated course of gestation and those with pre-eclampsia. It is undetermined whether homocysteine levels should be evaluated in all women with a history of serious pre-eclampsia in a previous pregnancy. Repeated studies in women with angiopathy and high homocysteine levels have proved that pre-eclampsia was seven times more frequent in their pregnancies when compared to those with normal homocysteine levels. Homocysteine does not appear to activate the endothelium as there was not found a comparison between fibronectin and homocysteine during the episode of pre-eclampsia. A study revealed that administration of folic acid and vitamins in women with a history of heavy early course pre-eclampsia decreases its frequency and severity in the subsequent pregnancy, but because these studies were quite small and not random, other greater ones are in progress. At the moment, no specific genotype has been associated with more severe or premature types of the disease. Therefore, homozygosity in C677T had an applicable pre-eclampsia risk of 2.6% (95% CI 1.4–5.1) and a hyperhomocysteinemia risk of 20.6% (95% CI 3.6–121.6) [90–95].

13.3 Hyperhomocysteinemia and recurrent abortions

High homocysteine levels have proven to be embryo toxic in guinea pigs through the vascular decidua and villi network destruction. Steegers-Theunissen and Co. found that between 8th and 12th week of pregnancy, there are high methionine levels and low homocysteine levels in the extra-embryonic cavity and amniotic fluid compared to the mother, suggesting that homocysteine accumulation may be toxic. Increased miscarriages in the first trimester are not linked to angiopathy, but to methyl group deficiency and defective DNA composition. Wouters and Co. were the first to notice the high hyper-homocysteinemia frequency in the 14% of cases with recurrent abortion with no prior normal pregnancies and in 33% of those with a history of normal pregnancy. Another study in 100 patients with a history of consecutive spontaneous abortions found homocysteinaemia in 12% of them, C677T of MTHFR mutation in 20%, and decreased folic acid levels in 15% of patients. Supplementation of high levels (15 mg) of folic acid and vitamin B6 (750 mg) to 28 patients with recurrent abortion cases improved homocysteine levels and the 17 pregnancies that followed had a successful outcome. In an after-analysis, homocysteine presence with or without methionine loading showed an increased hyper-homocysteinaemia risk by 4.2 and 2.7%, respectively. In another study where MTHFR genotypes were sought after in embryonic tissues and newborns, all genotype associations were found in embryonic tissue, while in newborns, there were no combinations of three or more mutant alleles. This reveals that embryos with a lot of mutations may be miscarried. As a result, it is proved that there is a correlation between homocysteine metabolic disorder and habitual abortions, but it is not clear whether administering vitamins before conception may prevent them [95–100].

13.4 Hyperhomocysteinemia and placental abruption

Placental biopsy in cases with abruption shows vasculopathy compatible with stenosis, necrosis, thrombosis, and atherosclerosis in the spinal arteries. Homocysteine in blood vessels acts by removing the methyl groups necessary for the DNA composition of multiplying cells. In many studies, hyperhomocysteinemia has been linked to placental abruption. In an after-analysis, folic acid deficiency was found to increase placental abruption frequency by 25.9% (95% CI-736.3) and hyperhomocysteinemia by 5.3% (95% CI 1.8–15.9). The presence of C677T of MTHFR polymorphism increases placental angiopathy risk by 2.45% (95% CI 1.00–6.02). In placental angiopathy cases, endothelium proteins are released in the blood, such as the von Willebrand factor (vWF), the activator of plasma tissue tPA (tissue plasma activator), the inhibitor of this activator PAI-1 (plasma activator inhibitor-1), fibronectin, and thrombomodulin that act as malfunction indicators. Women with hyperhomocysteinemia appeared to have a disproportionate tPA/PAI-1 ratio and a high vWF, while women with a placental abruption history had a high vWF and thrombomodulin. Thrombomodulin levels were in proportion with homocysteine levels. Administering antioxidant vitamins (folic acid, pyridoxine, and hydroxocobalamin) reduced the tPA/PAI-1 ratio but did not affect vWF, while the sole administration of folic acid reduced vWF levels. Combining hyperhomocysteinemia with thrombophilia, increases placental abruption risk by 3.4 [95–100].

Finally, angiopathy resulting from hyperhomocysteinemia may be at least theoretically involved in placental abruption, therefore it may be useful for cases with abruption in the future to be checked for thrombophilia and hyperhomocysteinemia and receive antioxidant vitamin treatment [95–100].

13.5 Other cases of hyperhomocysteinemia effects during pregnancy

Hyperhomocysteinemia may be associated with increased endometrial death by different mechanisms such as congenital disorders and pre-eclampsia, but its significance as an independent factor is in question. In a small group of patients, it was found to coexist with 11% frequency, while in a different group it was no more apparent than the rest of the population. The findings for its role in slowing intrauterine growth are contradictory. In another group of patients, hyperhomocysteinemia levels were as high as 38% and in a different group that was checked after methionine loading in women with a history of slowing intrauterine growth, hyperhomocysteinemia levels reached 19.2%. On the contrary, in a recursive review of the course of pregnancy in women who were CBS mutation carriers, newborns did not appear to have lower birth weight [95–100].

14. Hyperhomocysteinemia treatment

Treatment with vitamin B6, B12, and folic acid on its own or combined with other vitamins has been evaluated on small groups of patients with coronary artery disease and obstetrical complications and has been found to induce homocysteine levels and incidents by 30–50% in these groups. In other studies, vitamin C and E were given as antioxidant factors. The significance of adding aspirin or heparin in these groups still remains questionable, even though we can conclude that normalizing homocysteine levels should be enough, in order to achieve the therapeutic response. From the above-mentioned factors, we can estimate that even if there is no unanimity, it is within reason to check homocysteine levels in cases with a

history of NTD, pre-eclampsia, recurrent abortions, and perhaps in cases with placental abruption and endometrial death. It is valuable to look further into the results from administering vitamins in these cases [95–100].

15. Further investigations

We and other researchers have been searching for such predictive blood biomarkers of miscarriage. Macrophage inhibitory cytokine 1 (MIC-1), which is During the first trimester of gestation macrophage inhibitory cytokine 1 (MIC-1), which is presented in the syncytiotrophoblast and deciduas increases in serum and it is proposed to play an immunomodulatory role in the progression of the pregnancy. Pregnancy is considered as an ideal condition to study the regulation mechanisms of vascular growth under physiologic circumstances. Fetal vasculogenesis, angiogenesis, and vascular adaptation of the uterine circulation are one of a kind [101–104]. There is strong evidence bracing a close relationship between embryonic development and the state of vascularization of the chorionic villi. Normal chorionic villous vascularization is crucial for the normal development of pregnancy. However, it is not well known whether abnormal changes in utero-placental vascular development predispose to abortions [101–104]. The development of a normal functioning placental vascular network requires an important degree of coordination between various angiogenic and angiostatic factors and is exquisitely dependent on signals exchanged between these factors. Abnormalities in the development of placental vasculature may generate a number of gestational pathologies including miscarriages, intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), placental abruption, and preeclampsia. The importance of angiogenesis and angiogenetic factors in pregnancy is well known, and it has been proved that **chemokines and their receptors** are implicated in pregnancy and abortion, while cytokines and chemokines have a crucial part in controlling immune cells; these molecules are synthesized at the maternal-fetal interface where they have been implicated to play critical roles in the establishment and maintenance of pregnancy. In addition, they take part in other biological processes, such as cellular lymphoid organogenesis, and expression of adhesion molecules. The role of chemokines in angiogenesis during pregnancy has been experimentally demonstrated in cultures of leukocyte-free first trimester gestational decidual cells and in spontaneous miscarriage in mice, but the angiogenetic and angiostatic role of chemokines in the placental growth and decidua has not been well demonstrated [101–104]. These preliminary results propose a disturbance of the chemokine-associated angiogenetic network with a significant number of spontaneous abortions during the 1st trimester of gestation.

16. Conclusion

Miscarriage is the most usual complication of pregnancy. There are currently no definite predictive tests and treatments that can prevent spontaneous miscarriage. While 50% of miscarriages are associated with fetal chromosomal faults, most of the remaining cases are likely to be euploid fetuses that have failed due to implantation problems. Numerous investigators have previously figured that developing an accurate predictive test for miscarriage may open the window for identifying euploid pregnancies that are still viable but intended to miscarry. It follows therefore that possibly, emerging therapeutics could be targeted at such high risk euploid pregnancies so that some of them may continue to viability,

(i.e., rescuing some from miscarriage). The treatment of miscarriages and especially RPL has a wide variety and should be targeted at the reason. It could be complicated to recommend general therapeutic management especially if they are unproven, invasive, and expensive, because most couples with unexplained recurrent miscarriages have a good outcome. Further investigation of the factors that regulate the possible transcriptional repression of angiogenic chemokines and/or the overexpression of the angiostatic chemokines could cooperate in early prediction and prevention of spontaneous abortions. We assume that an accurate investigation of chemokine networks possibly taking part in the angiogenetic mechanisms of pregnancy would assist in the design of more accurate and possibly individualized angiogenesis-associated strategies for improving the early prediction and prevention of spontaneous abortion.

Author details

Panagiotis Tsikouras[1*], Theodora Deftereou[1,3], Xanthoula Anthoulaki[1], Anastasia Bothou[1], Anna Chalkidou[1], Fotini Gaitatzi[1], Ioannis Tsirkas[1], Arsou Chalil Bourazan[1], Eirini Bampageorgaka[1], George Stanulov[1], Elefterios Chatzimichael[1], Spyridon Michalopoulos[1], Panagiotis Petsidis[2], Georgios Iatrakis[4], Stefanos Zervoudis[2], Maria Lambropoulou[3], Werner Rath[1,5] and Georgios Galazios[1]

1 Department of Obstetrics and Gynecology, Democritus University of Thrace, Greece

2 Department of Obstetrics and Gynecology, Rea Hospital, Athens, Greece

3 Department of Histology-Embryology, University Hospital Alexandroupolis, Greece

4 Department of Obstetrics and Gynecology, University of West Attica, Greece

5 Department of Obstetrics and Gynecology, University of Germany, Germany

*Address all correspondence to: ptsikour@med.duth.gr

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