Pleading for the Routine Introduction in the Investigation of the Late Spontaneous Abortion Etiology of the Exploration of Resistance to Activated C Protein Along With Histopathological Placentary Screening

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Abstract: This study is an evaluation based on placental screening, of the importance of ascending intrauterine infection and placental vascular pathology. The paper is as well a plea for the routine use of the modern histopathological exam of the products of conception along with the noninvasive screening of chorioamnionitis (by means of serum C-reactive protein measurement) in the evaluation of late abortion (the recurrent one in particular) since the ascending intrauterine infection and placental vasculopathy seem to play an important part in the pathogenesis of this type of miscarriage. The therapeutic measures against this particular type of preterm labor should therefore focus on either bacterial vaginosis (the world most frequent cause of recurrent abortion of infectious origin) or/and thrombophilia even before the standard lab results are available.

Key-Words: Placental screening, Ascending intrauterine infection, Recurrent abortion, Vascular pathology of placenta, Late miscarriage

1 Introduction

Romero and his coworkers [1,2] and Arias with his team [3] have filled in, and Rai et al's studies [4, 5, 6, 7] and Spong et al's studies [8] have supported the valuable notion of the placental histopathology screening for the ascending intrauterine infection injury and for the placental vascular injury.

According to Burns group [9], the relation infection – recurrent abortion is an actual active research subject, in order to establish whether there is a connection between a predisposition inherited during the infection and the recurrent abortion (in the middle trimester also) or one can test for a strong association between specific groups of vaginal bacteria and the prognostic of pregnancy [1, 3, 8].

The vaginal infections role of causing preterm recurrent labor is a new area of research [10], which seems to target with predilection the bacterial vaginosis defined as alteration of the vaginal flora, when the number of lactobacilli that normally predominate is low or they are completely absent [11, 12]. As a consequence, the Hay group [13] reported that the presence of bacterial vaginosis in the first trimester of pregnancy is a preterm birth and late abortion risk factor, without showing though any association with early abortion.

The importance of bacterial vaginosis in the genesis of late abortion is emphasized by the evaluations performed on the natural history of bacterial vaginosis during pregnancy by the Llahi group [14].

Coagulation disorders are nowadays well known causes of recurrent abortion, characterized by defective hemochorial placentation and presence of microtrombs in the placental vascularization [15].

According to H. Marret and collaborators [16] the thrombophilia can be wined (primary and secondary anti phospholipid syndromes), congenital (the quantitative and qualitative constitutional deficits in

the natural inhibitors of the coagulating: C-protein and S antithrombin or movements that are impressing the target factors of those inhibitors and incapacitate their action: factor V Leiden and that of the prothrombin or the movement G202A10) [16]. To those it can be added some of the more complex anomalies such as hyperhomositeinemia.

Various components of the coagulation cascade and fibrinolysis are important in the embryonary implantation, the trophoblast invasion and placentation, which sustains the 20% value of late abortion in the group of recurrent abortions [4].

Pregnancy is a state of secondary hypercoagulability characterized by: (1) increase in the levels of the procoagulant factors (VII, VIII, X and fibrinogen), (2) decrease in concentration of the natural anticoagulants (anti thrombin III and C and S proteins) and (3) decrease in fibrinolysis (while the concentration of the plasminogen activators remains stationary the levels of inhibitors 1 and 2 of the plasminogen activators increase 5 times compared to the pre-labor status [17]).

The trophoblast invasion and the implantation seem to be controlled also by the balance between the plasminogen activators and the inactivators; their unbalance would generate defects of implantation and placentation, while the defective invasion of the spiral arteries proved to be a frequent pathological modification in the placentary biopsy fragments obtained from women who had abortions but also from those whose pregnancy had been complicated by preeclampsia and intrauterine growth restriction [18].

In case of a normal placenta, important components of the hemostasis, fibrinolysis and protein C dependent anticoagulation mechanisms are present and responsible for a correct distribution of the fibrin, while the abnormal pregnancies are characterized also by a bad fibrinous distribution [19]. A possible mechanism for this effect is the production of certain cytokines or the presence of endotoxin, which convert the endothelium from a thromboresistant surface to a thrombogenic surface [7].

It was postulated that a normal allogeneic reaction to the pregnancy initiates hemostatic and fibrinolytic reactions that ensure a stable implantation, while an inadequate allogeneic response determines a failure in the initiation of those actions, leading ultimately to interruption of the pregnancy [19].

As a support to this theory, there was observed sedimentation of fibrin in the chorionic villi that make an allogeneic contact to maternal tissues and in the villous areas containing T helper lymphocytes, activated macrophages, platelets, components and products of the hemostasis [20]. It has been proven that the endothelial cells of the villous areas from the chorionic villi lack the thrombin-thrombomodulin anticoagulant mechanism, unlike the endotheliocytes of the normal villi, possessing this mechanism [19, 21].

Rai and his coworkers [7] underline the fact that women with a history of recurrent abortion find themselves in a procoagulant state even outside of pregnancy, by means of fibrinolysis disorders (most frequently involving plasmatic excess of plasminogen activator inhibitor 1) that appear independent of and more often than the anti cardiolipin antibodies positive titres.

The same group of researchers [7] but also the Hauth team [22] have shown that hemostasis anomalies (either as an unbalance of thromboxane-prostacyclin ratio in favor of the thromboxane that can lead to vasospasm and trophoblastic platelet aggregation accompanied by the appearance of microthrombs and placentary necrosis or as a significant drop in the serum C protein concentration) precede with a number of weeks the abortion and that these modifications can be reversible by administering heparin, thus allowing the pregnancy to continue until its term.

Nowadays it is well demonstrated the influence of the immune maternal response in the genital infection with Ureaplasma urealyticum, responsible, among other things, of recurrent abortions and also of activating the polyclonal B lymphocytes with the increase of various antibodies, such as the anti phospholipin antibodies [23, 24, 25].

Besides the primary anti phospholipid syndrome which is responsible for 15% of all recurrent abortions, often manifesting late, by extended placentary thrombosis, because of the decrease in concentration of annexin V (phospholipid binding protein, with a role of powerful physiological anticoagulant) from the surface of the placentary syncytiotrophoblast [26], nowadays, other specific coagulation defects are admitted as generators of recurrent abortion: (1) resistance to activated C protein [6, 27, 28], (2) deficit of coagulation inhibitors, represented either by a low level of C protein (associated to late abortion via extensive placental infarction but also to preeclampsia and intrauterine growth reduction) or by a reduced concentration of the S protein (associated to fetal morbidity via massive placentary thrombosis [6]), (3) a low level of specific coagulation factors (such as activated factor XII which, by inherent insufficiency of fibrinolytic mechanism, causes thrombotic extensive placental infarction responsible

for late recurrent abortions), (4) primary thrombocythemia (generating both early as well as late abortions, but for which it is not clear how useful a therapy during a complicated pregnancy, with levels of serum thrombocytes of 800-1000x10^9/l, consisting of administration of interferon, hydroxyurea, plasmapheresis and antiaggregants is, because in absence of the therapy in case of pregnant women with primary thrombocythemia there were reported normal term deliveries in 100% of cases [7]), (5) anomalies of the homocysteine metabolism (amino acid resulted during conversion of methionine in cystine; congenital or developed hyperhomocysteinemia is associated with premature vasculopathy and extensive placentary infarction, responsible for around 21% of late recurrent abortions; because developed hyperhomocysteinemia unlike the congenital one – autosomal recessively transmitted, is in many cases the consequence of folate deficit, so a simple administration of folic acid in case of these patients brings the level of homocysteine to normal values in a matter of days [7]).

The resistance to the activated C protein is the thrombophilic defect the most studied nowadays, after the primary anti phospholipid syndrome, in the recurrent abortion genesis, that affects with predilection the second gestational trimester [27].

The C reactive protein resistance is autosomal dominant inherited and is recognized as being the most important cause of vein thrombosis (with a prevalence of 60% among people with vein thrombosis) and hereditary thrombophilia [6].

In over 90% of C reactive protein resistance cases the cause is represented by a single point mutation (Glutamine-Arginine) at the nucleotide from position 1691 of the coagulation factor V gene (Leiden mutation of factor V), and the factor V with Leiden mutation is resistant to inactivation through activated C protein (which under normal circumstances inactivates the Va and VIIIa factors in the presence of the cofactor – S protein) which leads to the generation of increased quantities of thrombin and implicitly to a state of hypercoagulability [4].

Starting from the data offered by the technical literature that draws attention on the placentary thrombotic etiology of the late abortion [20, 3, 6] as well as on the large prevalence of resistance to activated C protein among those affected by the venous thromboembolism, Rai and coworkers [7] have investigated the association between the resistance to activated C protein and late abortion, by screening, to find the resistance to activated C protein via Coatest (Cromogenix) coagulation test, 120 women with history of recurrent abortions (70 with first trimester recurrent abortions and 50 with late abortions) and a control group of 70 multiparous women without history of spontaneous recurrent abortions (none of the subjects investigated in this manner had history of thrombosis or were pregnant during the investigation or under influence of oral contraception or positive for lupus anticoagulant or anti cardiolipin antibodies – IgG and IgM).

The results of this investigation performed by the Rai group [7] show a prevalence of the resistance to activated C protein significantly larger among women with history of late recurrent abortion with respect to both women with repeated first trimester abortions and women from the control group. The authors conclude that these results suggest that the resistance to activated C protein can be an important mechanism of late abortion, possibly connected to intravascular hypercoagulability which is normal in pregnancy, knowing that resistance to activated C protein develops during pregnancy as part of the hemostatic normal modifications of the pregnancy, making the women with resistance to activated C protein, preceding the pregnancy, to develop a more pronounced resistance to activated C protein once the pregnancy is installed and develops.

The idea behind using the antiplatelet and antithrombotic treatment in case of women with recurrent abortions (frequently affecting the second gestational trimester) is based on the demonstration, in a large number of such cases, of the fact that the spontaneous miscarriage is due to the unbalance of the coagulation and fibrinolysis mechanisms, in favor of coagulation, with an excess sedimentation of the fibrin in the placenta [29].

Low doses of aspirin (60-150 mg daily) irreversibly inhibit the cyclooxygenase enzyme in platelets and macrophages, which converts the metabolism of the arachidonic acid towards the pathway of lipoxygenase and implicitly inhibits the thromboxane synthesis without affecting the production of prostacyclin (PGI2, eicosanoid discovered in 1989, vascular specific prostaglandin, produced mainly by the endothelium and in the cells of smooth vascular muscle and which can be considered one of the most powerful local vasodilators [30]) but with the appearance of an excess of leukotrienes, capable of stimulating the production of interleukin 3, which is important for implantation and promoting the placental growth [22]. The efficiency of low doses of aspirin to improve the prognostic of the pregnancy may therefore be due to the aspirin induced placental growth stimulation, sufficient to compensate the ischemic atrophy of the placenta and not only to the alteration of the thromboxane – prostacyclin ratio.

The heparin inhibits blood coagulation by means of two mechanisms [6]. In conventional therapeutic doses, heparin increases the inhibiting action of anti thrombin III on the activated factors XII, XI, IX, X and on thrombin. In large doses heparin catalyses also the inactivation of thrombin by the heparin cofactor 2. Neither the unfractioned conventional heparin nor the new low molecular weight heparins do not penetrate the placenta, but usage of both types associate the risk of both osteopenia which is reversible when the treatment stops as well as thrombocytopenia (idiosyncratic response) which can generate either bleeding or thrombosis and therefore the heparin therapy must be monitored by repeated count of thrombocytes [7].

In 1995, Wolf and Horger [31] focus the world's attention on the necessity of a standard investigation of the recurrent abortion (based on the progress of ultrasonography and tissue culture for the systematic chromosomal analysis of genitors and conception product that would bring significant savings, of thousands of US dollars per investigated case, alongside reduction of the stress and rapid solving of the case) that would comprise, besides the cytogenetic analysis of the parents and conception product, an anatomic study (hysterosalpingography and/or hysteroscopy and/or pelvic ultrasonography), endocrine evaluation (serum dosage in 8th day of the luteinizing hormone – LH, foliculostimulating hormone – FSH and testosterone), investigation of the autoimmune and infectious etiology (whose importance in inducing late abortion might be primarily connected to bacterial vaginosis [32]), recommending for this the following tests: complete blood count, activated partial thromboplastin time, dosage of anti cardiolipin and antinuclear antibodies, cervical cultures, hysterosalpingography, parental karyotype and cytogenetic analysis of the conception product. Rotmensch and coworkers [27] indicate the necessity of introducing in the investigation of the recurrent abortion of the repeated screening test for the activated C protein resistance, next to the molecular diagnosis for the Leiden mutation of the coagulation factor V.

The Rai group [6, 7] completes the list of thrombophilic defects exploration with dosage of anti thrombin III, of C protein concentration, of S protein (total and free), of factor XII, of fibrinogen, plasminogen and normality of fibrin plate lysis but also of congenital or developed hyperhomocysteinemia (rapidly correctable by supplements of folic acid) which although much rarer than anti phospholipin antibodies and resistance to activated C protein are nowadays more and more often associated to second trimester abortion by extensive placentary infarction. Dizon-Townson and coworkers [28] appreciate that 42% of placentas with over 10% of the surface being infarcted reflect the fact that the fetus carries the Leiden mutation of the factor V (the most frequent genetic predisposition to thrombosis, often marked by the thrombosis of the umbilical vessels along with extensive placentary infarction).

The importance of investigating the above mentioned thrombophilic defects that besides extensive placentary thrombosis affects the embryonary implantation as well as the placentation, was underlined by the Rai group [5], who drew the attention on the possibility of preventing there unfortunate effects by adjusting the aspirin-heparin association, already successfully used in treating the anti phospholipin syndrome. Moreover the Rai team [5] suggests that the efficiency of preventing recurrent abortion, besides using antiplatelet and antithrombotic drugs, is connected to the antibiotic treatment of the bacterial vaginosis (in case of late recurrent abortion of infectious origin) but also very likely to the modern therapy for polycystic ovary syndrome with gonadotropin releasing hormone agonists (in cases with elevated LH), and to the judicious application of the cervical cerclage in the cervico-isthmic incompetence (usually overrated diagnose [33, 34, 35, 36, 37]), besides the systematic indication of karyotyping the conception product with genetic advice when needed. The list of preventing the recurrent abortion is completed by educational measures regarding the reduction of weight excess as alternative measure to the administration of insulin antiresistance drugs in the polycystic ovary syndrome [38], but also smoking and alcohol consumption among women at procreating age, social factors that dramatically increase the risk of late abortion, preterm labor and many more [32] – easily detected therefore at a low cost, by means of a correct anamnesis and which adversely influence the function of the human hemochorial placenta.

The justness of directing the prevention of recurrent abortion (especially for the second trimester) observed in the studies of the Rai group [5, 29] is remarked also from the many morphopathological observations of the conception product that are systematically detecting, but in various proportions, in the late miscarriage and even in the preterm births (that are overlapping, between 20-28 gestational weeks [39, 40]), the placental ischemia and/or the acute amniochoriodecidual inflammation, as the most often involved pathological processes in the multifactor etiology of the preterm labor, slightly understood [1, 2, 8, 20,

24, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54].

Arias and his coworkers [3], according to Spong and his coworkers [8] are mentioning that 70% of preterm labors, with or without premature rupture of membranes, are the result of ascending intrauterine infection and placental vascular pathology. And if very large studies will confirm these observations, that means that desperate efforts to inhibit premature uterine activity, well installed, in order to prolong the pregnancy, are unnecessary not only because of the lack of efficiency (according to Rust et al [55] that shows that in advanced labor the conventional tricolysis is inefficient), but also because the extended pregnancy keeps the fetus in a hostile environment, that more is harming than supporting.

Like Arias group [3], Rai and his coworkers [6, 7], and also Dizon-Tawson and his coworkers [30] are suggesting that the histopathological examination of placenta, which reveals a placental vascular pathology like placental infarction expanded to over 10% of the surface, with or without calcifications (Rand et al [26]) and/or accelerated streaking of the placenta villi next to the multiple syncytial nodules or chorioangiosis placenta , possible the absence of adaptation of the spirulate decidua arterioles is a practical screening in recurrent undue abortions,preterm labor and the delay growth or fetal death in the uterus.

The groups Arias [3], Rai [6, 7] and Dizon-Tawson [30] in agree with the observation of the arterial flux utero and fetal-placental trough ultrasonography [56], agree with the possibility that the deficiency of the trophoblast could generate changes in the spirulate arterioles, which is the cause of unequal and improper utero-placental blood flow which will be the basis of accelerated maturation, with numerous syncytial nodules and fibrosis of the placental villi and at the same time of the placental attacks, by diverse intensity, knowing, on the other hand, that the thrombophilic issues could generate extended placental thrombosis, or placenta defects and embryonic implantation issues [7].

Is not clear the nature of the link between the absence of an appropriate hemochorial placentation and such a varied clinical expression, from the pre-eclampsia to the delay growth or fetal death in uterus, or preterm labor or premature rupture of membranes and recurrent abortion in the second trimester of pregnancy [3].

Our retrospective clinical-morphopathological study of the miscarriage in “Filantropia” Maternity from Craiova, was made in light of modern anathomopathological screening of the concept product in an attempt to find explanations for the emergence of extremely premature labor and the extremely premature rupture of membranes (in the second trimester of pregnancy) and eventually in finding out solution to prevent them by comparing the results of clinical-morphopathological exam with the evaluation of women who have a normal pregnancy and gave birth at term.

2 Material and Methods

We have studied 59 placentas from late miscarriages between 14 - 27 gestational weeks in the “Filantropia” Maternity from Craiova, excluding multiple pregnancy and fetal malformations. The 59 placentas were analyzed retrospectively clinical-anatomopathological.

We have used for control the clinical-morphopathological data from 40 normal pregnant women who gave birth at term, because the majority of miscarriages studied occurred between 20-27 weeks of gestation, so they can be framed in the category of extremely preterm births [39, 40].

Clinical information was obtained from case report. It includes demographic data, gestational age, obstetrical history, health status at admission and laboratory investigations. In the studied group of 59 late miscarriages were recorded as associated diseases: recurrent abortion in 56 cases and premature rupture of membranes, in less then 24 hours, in 35 cases.

The morphopathological examination of the placentas was performed by the pathologist without knowing the clinical details of the case following the Gaillard [57] protocol, which can be summarized: placenta fixed in buffered formalin 10% was weighed, measured and then sectioned for taking fragments from all its thickness, from the extraplacentary membranes and from the umbilical cord to put it in paraffin .That preceded the microscopic study of the sections stained with hematoxylin-eosine or Van Gieson from the fragments above mentioned.

Histological,chorioamniotitis was characterized by polymorphonuclear leucocytes, with or without necrosis present in the fetal membranes and subcorial fibrin [3].

The severity of the polymorphonuclear infiltrate (Fig. 1, 2, 3) was based on the number and the degree of infiltration of neutrophils, their marginal and mixed choriodecidual disposal, vascular umbilical disposal and in chorial board. Was also based on the presence of the limphoplasmocitary infiltrate with or without vascular degeneration of the fetal membranes, sometimes even with

morphopathological signs suggesting placental insufficiency [1, 2, 3, 20, 24].

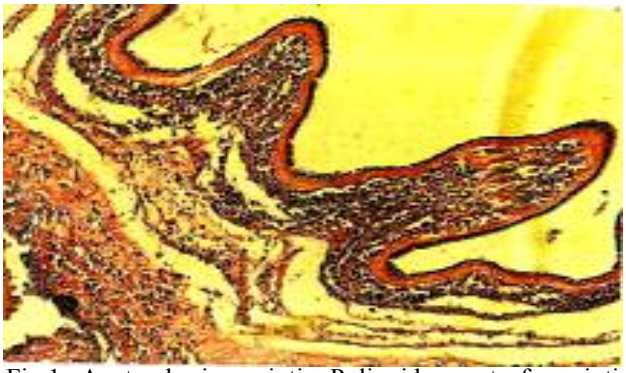

Fig.1 Acute chorioamniotis. Polipoid aspect of amniotic membrane with rich inflammatory exudates in the chorioamniotic axe; edema and exudates in the capsular deciduas- the severity of the amniocoriodecidual granulocyte reaction suggests just like the acute marginal coriodecidual inflammation the ascending intrauterine infection (Hematoxylin-eosina; ob.10)

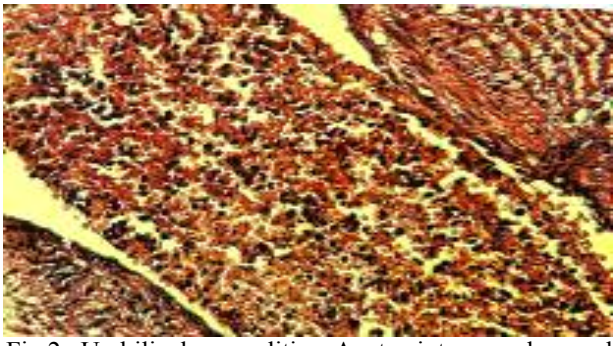

Fig.2 Umbilical vasculitis. Acute intravascular and intraparietal inflammatory exudates, parietal edema. Umbilical vasculitis, as funiculitis, is an indicator of maximal specificity (higher positive predictive value)of microbial invasion of amniotic cavity (Hematoxylin-eosina; ob.20)

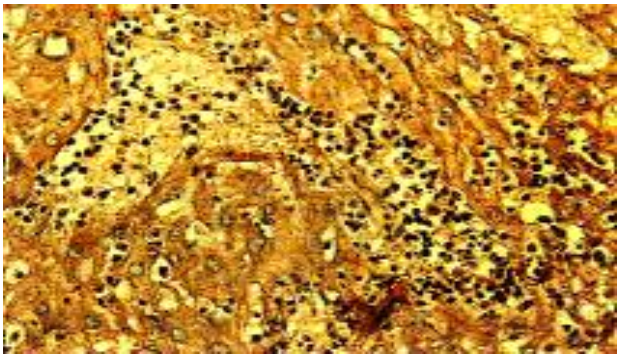

Fig.3 Acute inflammation of the corial board (Acute placentitis) is the most sensitive indicator (higher negative predictive value) of microbial invasion of amniotic cavity. Vacuolar decidual dystrophy with the inflammatory limfoplasmocitary exudates focal suggests the invasion of the amniotic cavity with Ureaplasma urealyticum (Van Gieson; ob.20)

The sever maternal and fetal placental vascular pathology was diagnosed [26, 28] by the presence of many placental infarctions (exceeding 10% of placental surface) in addition with either umbilical vein thrombosis (Fig.4) or suggestive calcification of aggression of the antiphospholipin antibodies type IgM (Fig.5) and the uneven accelerated maturation of chorionic villi, sometimes associated with multiple multinucleated syncytial nodules (Fig.6).

A mixed lesion consisted in a coexistence of taint with maternal and/or fetal placental vascular pathology [3].

Fig. 4 Umbilical vein thrombosis. Endoteliosis lesions.Parietal edema (Van Gieson; magnifier)

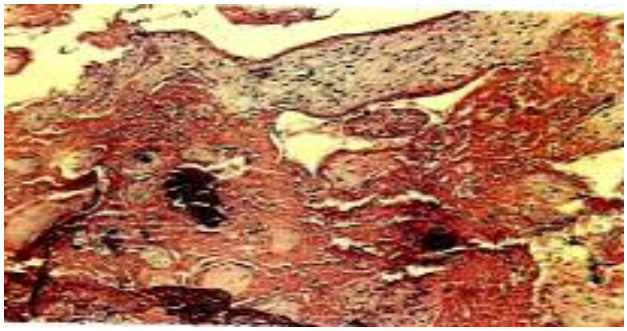

Fig.5 Placental infarction. Fibrinoid necrosis near the atrophic vilosities with intens sclerohialinization focus, marked calcification – the last suggesting the possible aggression by antiphospholipid antibodies type IgM (Hematoxylin-eosina; ob.10)

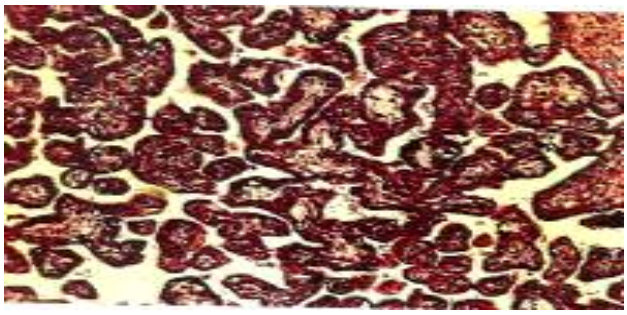

Fig. 6 Old placental infarction. Big areas of accelerate aging of chorionic vili that appear abnormally small, avascular, with intens sclerohialinization. (Hematoxylin-eosina; ob.10)

Chronic villitis / hemorrhagic endovasculitis were characterized by chronic inflammation areas with mononuclear cell infiltration, while fibrinoid necrosis areas affected groups of vilosities and the granulocytes were missing. They were also characterized by hematic intravillous infiltration with vascular villous parcellar edematous dystrophy [20].

Abruptio placentae was diagnosed when a retroplacentary thromb left a final impression on the maternal surface of the placenta, impression that is histopathological obvious [3].

A value of $p<0,05$ had a statistical significance at the comparing the results of the Mann Whitney U test and λ (chi square) test – with the Fisher correction for the small groups [58].

3 Results and Discussions

The descriptive variables (demographics) for the patients of the three groups (late miscarriage with intact membranes, late miscarriage with premature rupture of membranes and the control group), clinical-morphopathological evaluated, are presented in Table 1.

Table 1 The demography of the patients with late miscarriages versus group control

Characteristics	Abortion with intact membranes No.=24 (average +/- standard error of the average)	Significance (Mann Whitney U test)	Control group No.=40 (average +/- standard error of the average)	Significance (Mann Whitney U test)	Abortion with premature rupture of membranes No.=35 (average +/- standard error of the average)
Age (years)	23 +/- 0,82	Insignificant	25,6 +/- 4,3	Insignificant	25 +/- 2,4
Parity	0,12 +/- 0,1	Insignificant	0,23 +/- 0,15	Insignificant	0,1 +/- 0,1
Gestational age (weeks)	23,2 +/- 2,1	P< 0,05	39,02 +/- 1,2	P< 0,05	24,1 +/- 2,3

Table 2 The classification of the patients with late miscarriages made according to the clinical-morphopathological results

Results	Miscarriage with intact membranes No.= 24	Significance (chi square test)	Control group No.=40	Significance (chi square test)	Miscarriage with premature rupture of membranes No.=35
Ascending intrauterine infection	5 (20,8%)	P<0,05	4 (10%)	P<0,05	9 (25,7%)
Placental vascular pathology	16 (66,6%)	P<0,05	5 (12,5%)	P<0,05	18 (59,3%)
Mixed infectious and vascular lesions	1 (4,1%)	Insignificant	2 (5%)	Insignificant	3,8 (8,5%)
Chronic villitis/ Hemorrhagic endovasculitis	0 (0%)	Insignificant	1 (2,5%)	Insignificant	1 (2,8%)
Abruptio placentae	1 (4,1%)	P<0,05	0 (0%)	Insignificant	0 (0%)
Without previous placentary lesions	1 (4,1%)	P<0,05	28 (70%)	P<0,05	4 (11,4%)

As expected, we can notice a significant difference (Mann Whitney U test, $p<0,05$) between control group and the groups we have studied (late miscarriage with and without premature rupture of membranes) only in terms of gestational age at which the conception product was expelled. That means that for the three groups there are equal risks of infection and placental vascular pathology.

Table 2 presents the classification of patients in the following categories: infection (ascending intrauterine infection), placental vascular pathology, mixed lesions, chronic villitis/hemorrhagic endovasculitis, abruptio placentae and normal aspect (without previous placental lesions), according to the definitions set in subsection "Materials and methods" and partially exemplified in Fig 1-6.

Table 2 shows that:

a) The prevalence of intrauterine infection and placental vascular pathogeny was significantly higher at patient with late miscarriage with intact membranes and premature rupture of membranes compared to control group who gave birth at term.

b) Abruptio placentae is significantly more common at the patients with late miscarriage compared to control group.

c) The number of patients with normal aspect of the placenta (without the lesions in Table 2) was higher in the control group compared to the group with late miscarriage with intact membranes and premature rupture of membranes.

d) Chronic villitis and hemorrhagic endovasculitis were not so common in the control group and in the groups we have studied.

e) Surprising was the observation of the prevalence of ascending intrauterine infection, which was significantly undefined between the group of patients with late miscarriage and intact membranes and the group of patients with late miscarriage and premature rupture of membranes. This observation is supported by similar results of the Salafia group [20] and indirectly by Gaillard and his coworkers [57]. They find out that more then half of the 420 patients who had miscarriage in the second trimester of pregnancy with intact membranes had the amnioculture positive.

f) Also, the two groups studied with late miscarriage and premature rupture of membranes or intact membranes are not significantly different in terms of incidence of placental vascular pathology which allows a joint evaluation of the two studied groups. The evaluation indicates on the population with late miscarriage with premature rupture of the membranes or intact membranes a 57,6% prevelance of placental vascular pathology, and a 23,7% prevalence of ascending intrauterine infection. That means that the etiopathogeny of late miscarriage, or in other words of very preterm birth (only 11,2% of patients with late miscarriage were under 20 gestational weeks) from "Filantropia" Maternity from Craiova is dominate by the placental vascular pathology and ascending intrauterine infection in 81,3% of cases. This value is close to 71% found by the Arias group at the St. John's Mercy Medical Center, St. Louis [3], by adding the placental vascular pathology cases (with incidence around of 34% regardless of status membranes) with those affected by ascending intrauterine infection (with incidence of 37% regardless of membranes status). They have been verified on basis of standard criteria clinical-anathomopathological (similar to those used in this evaluation) and microbiologically (cultures of placental tissues) examed among the 105 cases of preterm birth.

The domination of the etiopathogeny of late miscarriage and preterm birth by the placental vascular pathogeny and the ascending intrauterine infection noted here is in agreement with the results of other international groups [8, 20, 42]. Study findings, among the 59 late miscarriage with or without premature rupture of membranes, of a 23% prevalence of ascending intrauterine infection is similar to that identified by assays of C-reactive protein in a group of 58 patients with miscarriage in the second trimester of pregnancy who were interned in obstetrics-gynecology clinics of the University of Medicine and Pharmacy from Craiova [59, 60]. This argues in favor of the current study, apparently limited number and selective (59% premature rupture of membranes,>90% recurrent miscarriages), and on the other hand recommends the dosage of CRP in the second trimester of pregnancy as a non-invasive screening, fast and not expensive, of high urogenital infection which allow the tempest application of modern therapy in a preterm labor of infectious origins.

The research of Quinn's group [23] is revealing the fact that the human infection with Ureaplasma urealytium is capable to induce the presence of antiphospholipid antibodies which are responsible for thrombotic placental lesions [26].

There were found histopathological lesions with Ureaplasma urealytium [24] in the sections of 4 cases with vascular pathology of the placenta, cases that can be incorporated in the population with ascending intrauterine infection.

The incorporation of the four cases of placental vascular lesions and all mixed lesions from our statistic in the ascending intrauterine infection is correcting the 23,7% and 57,6% prevalence, allowing in this way an almost perfect overlap

between the incidence of the ascending intrauterine infection and vascular pathology of the placenta on the population with late miscarriage (very preterm birth) investigated here with the one observed by Arias group [3] on a group almost double of preterm births (including the very preterm births).

This overlap is a confirmation of the power of this study and the validity of its results.

This study of late spontaneous abortions supports, alongside other investigations [4, 5, 6, 7, 8], the valuable notion of placental histopathological screening for ascendant intrauterine infectious lesions and placentary vascular lesions [1, 2, 3], by observing on one hand, the severe polymorphonuclear infiltrate of the chorial plaque, the marginal and mixed acute inflammation of the choriodecidua and umbilical vasculitis in respectively 100%, 80% and 20% of cases labeled as acute chorioamniotitis (ascendant intraamniotic infection), and on the other hand the extensive placental infarctions on more than 10% of the villous area in approximately 50% of the vascular placental lesions out of which 6% suggested the persistent presence of anti phospholipin IgM antibodies and approximately 12% of the extensive placental thromboses associated with umbilical vein thrombosis, very suggestive for Leiden mutation of fetal factor V [28]. For the rest of approximately 50% of vascular placentary lesions others than extensive thrombosis, given the composition of the investigated group of over 90% recurrent abortions, the probable causes of vasculopathy are: thrombophilic defects (knowing that these are determining venous thromboses and placentation defects [7]), endocrine factors, immune factors, chromosomal structure defects or/and uterine malformations [32, 61].

Because of over 42% of extended placental thrombosis are caused by the Leiden mutation of the V factor [28], the most frequent genetic predisposition to vein thrombosis and because the placental vascular pathology has affected almost 50 % of cases we can say that this study is an argument for introducing the placental screening in the investigation of the ethiology of late miscarriage .It is also an argument for adopting an early exploration of the C-reactive protein resistance which once detected in a patient with abortion, especially iterative one, will impose the exploring of her relatives, which will increase efficiency, not only of iterative abortion, disprove by starting the antithrombotic therapy before conception [6, 7], but the risk of thrombosis in general population.

If another study of the same type presented here, but larger, of miscarriage, especially iterative one, will confirm at the population of “Filantropia” Maternity from Craiova, the dominance of ascending intrauterine infection and the placental vascular pathology in the ethiopathogeny of preterm labor, in the condition of excluding the possibility of placental vascular pathology in the asymptomatic phase of the ascending intrauterine infection by the CRP serum screening. [59, 60] will appear the possibility of increasing the efficiency of the first prophylactic and curative measures in the extremely preterm labor before obtaining the histopathological examination results from the previous miscarriage.

This evaluation draws the attention to the large presence of subclinical chorioamniotitis, because none of the cases with positive histhology of chronic chorioamniotitis (according to Romero group [1, 2]) investigated here, had no fever, maternal tachycardia or leucocytosis.The high frequency of subclinical chorioamniotitis is not something new [1, 2, 3, 20].

4 Conclusion

This study is an evaluation based on placental screening, of the importance of ascending intrauterine infection and placental vascular pathology (the thrombophilya has a central place). They appear as dominant (over 80% of the cases of late miscarriage) in the ethiopathogeny of late miscarriage (preterm births).The study draw the attention to the necessity of the rutine application of the modern histopathologycal examination in the investigation of the miscarriage in the second trimester of pregnancy. It also reiterates the importance of another non-invasive screening - CRP serum screening. So the first therapeutic and curative measures of extremely preterm labor can be orientated to combat bacterial vaginosis, trombophilia with the CRP-serum level normal or higher.

The study draws the attention also to the application of modern evaluation of iterative abortion. Because the placental vascular pathology is more common than the infection is necessary to introduce the exploration of the C-reactive protein resistance (the most frequent genetic predisposition of thrombosis).

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