# The infectious etiology of second trimester spontaneous abortion reflected in the peripheral blood

Carmen A. Bulucea, Nikos E. Mastorakis, Mariana F. Paun, and Alina D. Neatu

***Abstract*—**The infection's role in causing or facilitating preterm labor and abortion in the second trimester is nowadays increasingly described. The goal of this study was to quantitatively measure the serum CRP by immunoturbidimetric technique to establish the serum CRP concentration in the second trimester of uncomplicated pregnancy and the sensibility of this parameter in complicated advanced pregnancies. This prospective and controlled study was conducted on 75 pregnant, non smoking women with a gestational age between 13 and 27 weeks, checked in the sections of the Obstetrics and Gynecology Clinic of the University of Medicine and Pharmacy Craiova, whose serum CRP was dosed in parallel with leukocytes counting. The patients were split in several study groups including a control group. The data analysis indicates the lack of a positive correlation between serum CRP concentration and gestational age in the second trimester of normal pregnancy after 20 weeks. The results indicate a significant increase of the serum CRP concentration (but not leukocytes number) at 24 hours postabortum followed by a progressive decrease of at least 18% at 48 hours after second trimester uncomplicated abortion. Only the pregnancy complicated by urogenital bacterial infections is accompanied by pathological values of serum CRP significantly elevated with respect to those in a normal pregnancy or one complicated with threatened abortion of unknown cause and non-evolutive, or prior recurrent abortions. 72% of pregnancies complicated with urogenital bacterial infections are accompanied by pathological values of serum CRP (but not of the leukogram), while 100% of pregnant women with serum CRP concentrations $\geq$ 2 mg/dl were diagnosed with chorioamnionitis or acute pyelonephritis. The obtained results suggest that a repeated measurement of the serum CRP concentration could be a valuable predictive marker for intrauterine infection.

***Keywords*—**CRP, intrauterine infection, second trimester pregnancy.

## I. Introduction

THE consequences of preterm labor (including the extremely preterm and coinciding with the period between 20 and 27 weeks of pregnancy from the classical definition of late abortion [1, 2]) are among the most expensive and morbid ones in modern obstetrics [3, 4, 5].

Ideal for preventing preterm birth would be preventing the preterm labor [1] that depends on the identification of preterm labor risk factors [6].

Among the preterm/extremely preterm labor risk factors today there are mentioned: prior preterm birth and abortion, multiple pregnancy, a small weight before conception and a limited increase in weight during pregnancy, age of the pregnant woman under 20 and over 35 years, stress, hard physical work, prolonged orthostatism as well as inferior socio-economic status and alcohol ingestion [7, 8, 9, 10]. Unfortunately, the risk scores systems based on these factors present low sensitivity as well as low predictive value [11, 12].

Because the prediction and the prevention of preterm labor (including the extremely preterm labor) are not yet possible, the early and exact emphasize of the preterm contractions becomes extremely important, allowing the tocolytic therapy and the pregnant women to whom this is recommendable for greater success chance or at least the early warning gives a longer period of time for the pulmonary maturation therapy and the transportation in a well equipped medical facility [1], this because the interleukin 10 (IL-10 or the cytokine synthesis inhibitor [13, 14, 15]), the cyclooxygenase-2 specific inhibitors and the oxytocin antagonist (atosiban [1]) although very efficient tocolytics are still in a research stage and the usual tocolytics, not being uterospecific, in case of a real preterm labor cannot delay the birth/late abortion for more than 48 hours [16, 17].

Nowadays, research is intensely conducted on markers that allow an early detection and even the prediction of preterm labor, such as: the amniotic fluid endothelin [18, 19], interleukin 6 (IL-6) in vaginal secretion [20], in the amniotic fluid [21] or in maternal [22] or fetal (sampled by cordocentesis [23]) serum, the plasma ferritin [24] that, like the neonatal nucleate haematids number, correlates with the bacteriologically and respectively histologically [25, 26] proven chorioamnionitis, the maternal serum long chain fatty acids [27], the maternal serum placenta alkaline phosphatase [28], the soluble receptors of Tumor Necrosis Factor (TNF) in maternal plasma and amniotic fluid in second trimester of pregnancy [29], oxytocin and maternal serum oxytocin activity [30], maternal plasmatic concentration of Mg, Cu, Zn, Ca [31],

C. A. Bulucea is with the University of Medicine and Pharmacy of Craiova, Obstetrics and Gynecology Department, Romania (e-mail: abulucea@gmail.com).

N. E. Mastorakis is with the Military Institutions of University Education - Hellenic Naval Academy, Greece (e-mail: mastorakis4567@gmail.com)

M. F. Paun, is with the University of Medicine and Pharmacy of Craiova, Obstetrics and Gynecology Department, Romania.

A. D. Neatu is with the University of Medicine and Pharmacy of Craiova, Obstetrics and Gynecology Department, Romania (e-mail: aneatu@gmail.com).

Dopplercolor and transvaginal pulsatile markers [32], the angiogenin from the amniotic fluid in the second trimester of pregnancy [33], defensin 5 in the reproductive tissues [34], beta-endorphins in the fetal membranes and placenta [35], circulatory placental gonadotropin-releasing hormone (GRH) and corticotrophin-releasing hormone (CRH) and the serum CRH binding protein, as well as endometrial stromal CRH with respective messenger ARN [36, 37, 38], inflammatory cytokines in the amniotic fluid (IL-1, IL-6, TNF-α [39, 40]), expression of $24p_3$ lipocalin by the myometrium in labor [41].

But the most studied in the last years, with a statute of early diagnostic markers rather than predictive markers, have been the cervical and uterine activity measurements [42] and especially the oncofetal fibronectin from the cervical or vaginal mucus [43, 44, 45, 46, 47, 48, 49, 50 – evaluating the cervical-vaginal fibronectin in parallel with cervical echography, 51, 52, 53 – also in tandem with cervical echography], which, although the most specific marker today to identify the preterm labor potential, used as screening test between 24 and 36 gestational weeks has a negative predictive value of only 25-30% [53], imposing supplementary studies on existing markers, but also identification of other parameters for early detection and maybe even preterm labor prediction, having also practical qualities like: reduced price, noninvasive and simple technique.

One major hurdle in the diagnosis and efficient therapy of preterm contractions, as well as of early stages of late abortion (from the moment of association of extremely preterm contraction, between 20 and 27 weeks of pregnancy or extremely extremely preterm, under 20 weeks of pregnancy) is their heterogeneous and unclearly defined etiology [54, 55].

The infection's role causing or facilitating preterm labor and second trimester abortion (with painful contractions, extremely or extremely extremely preterm) has been frequently described [56], because it has been already objectivated that the infectious chorioamnionitis is present in 100% of second trimester abortions with non macerated dead fetuses [57], and over 65% of second trimester abortions and over 76% of preterm births recognize both bacteriologically and histopathologically the ascending infectious etiopathogeny via intact membranes favored by various degrees of associated cervical dilation [55, 58, 59, 60, 61, 62, 63].

71% [64, 33] – 81% [65, 66] of preterm labors, with or without premature membranes rupture, are the result of ascending intrauterine infection and placental vasculopathy, values reached by summing the placental vasculopathy cases, having an incidence of around 34% [64] respectively 57,6% [65, 66] with those affected by ascending intrauterine infection, with an incidence of 37% [64] respectively 23,7% [65, 66].

Gaillard and collaborators [55], based on the maceration degree of the conception products (420 cases analyzed) have isolated mophopathologically, microbiologically and clinically 2 groups of mechanisms of spontaneous second trimester abortion; (a) a group with no prolonged intrauterine retention (constituting 78% of cases), to which in 85% of late abortions there was found only one explanation of the spontaneous pregnancy termination, represented by ascending infection via unbroken membranes (chorioamnionitis); (b) the second group of conception products, severely macerated, allowed establishing the cause of fetal death in only 44% of cases, in which fetal anomalies and maternal factors predominated.

The same Gaillard team [55] shows the acute vilitis has been rarely encountered and of weak intensity among the 420 late spontaneous abortions complexly analyzed, excepting the case of specific infection with Listeria monocitogenes (micro abscesses) or candidosis or cytomegalovirus.

In case of one macerated dead fetus, Schwartz and co-authors [67] have identified the infection with cytomegalovirus, clinically not obvious, by means of optical microscopy, due to cellular specific viral inclusions, but in the context of severe necrosis it was only the electronic microscopy that could confirm the presence of the viral infection.

Rudbeck-Roge & Henriques [60], via a clinic, histopathological and microbiological examination of the expelled conception products following a second trimester spontaneous abortion, show that the subclinical chorioamnionic acute inflammation has been emphasized in 58% of cases, but the standard positive culture confirmed the criteria, unclearly formulated, of the anatomopathological diagnosis, in just 36% of cases.

Moreover, based on vague anatomopathological criteria of ascending intrauterine infection (chorioamnionitis), in case of a group of spontaneous abortions with dead fetuses, mostly less than 20 weeks of pregnancy, Ornoy and team [57] identify the infectious origin of the fetal death in 95% of cases.

Fedotova and Shastina [68] emphasize in half of the cases diagnosed with subclinical intrauterine infection and late spontaneous abortion Ureaplasma urealyticum as etiologic agent that would generate chorioamnionic characteristic lesions, such as vacuolar degeneration, limphoplasmocitary focal infiltrates and chronic placental insufficiency.

Labarrere and team [69] show that in normal placentas the areas of chronic vilitis and hemorrhagic endovasculitis (an increased frequency would suggest the presence of infectious / immunologic processes [70]) are rare.

Puggina and co-authors [71] find the placenta chorioangioma frequently associated to fetal malformations, polyhydramnios and preterm labor, while Horn and team [72] were underlining that the chromosomal aberrations imply structural distortions of the chorionic villi besides characteristic morphological alterations of the embryo or fetus.

Stepanov and collaborators [73] indicate the fact that the chronic placental insufficiency significantly alters the histogenesis and fetal thyroid function, evident signs in late spontaneous abortion.

Miloratov and Kurik [74] find after histopathological examination of non villous cytotrophoblast that both its volume and function reach a peak between 23 and 27 weeks in

normal pregnancy, followed by a gradual decrease until the term of the volume and function of cellular islands, septua and basal lamina in parallel with a progressive accumulation of intervillous fibrinoid with pseudo infarctions.

Salafia and co-workers [70] observe that, at term, the presence of an acute inflammation of the umbilical cord, amnion and choriodecidua, as well as acute severe inflammation of the chorial plate are responsible for bradycardic routes, on the electronic recordings of fetal cord rates. The infection of the amniotic fluid by ascending mechanism would alter the fetal metabolism, stated by the same authors, either by means of effects on the pulmonary and gastro-intestinal systems or effects on umbilical and chorionic vessels.

The Bernal [75] and van der Elst [76] groups demonstrate that the extremely preterm labor seems to be frequently caused by acute chorioamnionitis by means of increased production of prostaglandins (PG) in the amnion and choriodecidua, as a response, for instance, to the bacterial phospholipase activity.

Bernal and co-workers [75] show also that prevention of preterm labor complicated with chorioamnionitis is difficult, due to the fact that numerous facilitating medical and socio-economical factors surpass the obstetrician's control possibilities, suggesting also that the early diagnose of chorioamnionitis in case of pregnant women with preterm labor, via rapid and precise tests, that should be developed (for instance, CRP [77]) followed as early as possible by an aggressive treatment combining an efficient antibiotic and a strong anti-inflammatory with no side effects (for instance inhibitors of inducible cyclooxygenase [1]) could stop the preterm labor with a considerable improvement of the perinatal mortality.

The same Bernal group [75] indicate the possibility of preventing the preterm labor that is not complicated by chorioamnionitis by means of drugs that decrease the sensitivity, probably increased, of the myometrium to endogenous agonists, such as the oxytocin.

Any severe infection can cause sporadic spontaneous abortion [78, 79, 80, 81, 82, 83, 84, 1].

In order for a microbial agent to generate repeated pregnancy interruption, this either has to persist in the genital tract a long period of time or not cause bothersome symptoms to the patient [85]. Some chronic infections have been incriminated or strongly suspected of producing abortion [86].

Herpes simplex has been associated with an increased abortion incidence if the genital infection appears in the first half of the pregnancy or if the pregnancy happens in the first 18 months from the debut of a primary genital infection [85, 86].

The syphilis is due to Treponema pallidum, which can cross the placental barrier and threaten the fetus starting with the $16^{th}$ week of amenorrhea [86, 87].

In accordance with H. Marret and co-workers, in case of rubella, the first infection presents an increased fetal risk, with 80-100% serious malformations. Re-infection is possible, the fetal risk is not null, but probably very low [88].

Delcroix and Gomez state that the rubella virus can induce serious fetal anomalies just in case of first infection before 3 months of pregnancy. After 16 weeks of amenorrhea there is no longer any risk of fetal malformation. In case of maternal first infection between 2 and 12 weeks of amenorrhea the malformation risk is increased (50-80%). Between 12 to 19 weeks of amenorrhea the risk of fetal infection is estimated at around 15%. After 20 weeks of amenorrhea there are no indications of prenatal diagnostic of fetal rubella infection [87].

The hazard of chickenpox exists just in case of first infection and related to the pregnancy age. Before 20 weeks there is a malformation risk in case of first infection [89, 87]. There is the possibility of chickenpox reactivation as zoster, but the maternal zoster presents no risks to the fetus [89].

Munteanu and collaborators state that there is no clear proof to show that Toxoplasma gondii, Listeria monocytogenes and Chlamydia trachomatis can determine an abortion [86].

Merger [90] emphasizes the fact that the risk of abortion in toxoplasmosis exists only in case of a first infection, toxoplasmosis not being able to induce recurrent abortions.

In accordance with Delcroix and Gomez, the infection of the fetus in case of listeria is possible by means of a hematogenous placental with risk of abortion, premature birth or fetal death in uterus and also neonatal infection [87].

In case of a pregnant woman, low genital infections with Chlamydia trachomatis increase the risk of preterm birth, of fever during labor and postpartum endometritis. It is frequent a co-infection with Ureaplasma urealyticum [87].

Quinn and his team [91], following serologic observations and erythromycin based therapeutic success, emphasize the role played by Ureaplasma urealyticum in the genesis of recurrent abortions, cases in which there were revealed uterine cultures positive for Ureaplasma urealyticum in a proportion significantly larger than in case of the control group, and therapy, in between pregnancies, of affected women and their sexual partners, with doxycycline, would improve the reproductive prognosis of the couple.

Vokaer [79] states that rickettsioses and neo- rickettsioses determine abortions only during the second trimester of pregnancy, abortions that may repeat, the microorganism being able to infect again, when the placenta starts its functions, due to its tropism for the trophoblast.

Pertaining to the cytomegalic inclusion body disease, non apparent affection, affecting 60-80% of the population, the feto-placental contamination is always serious and a first infection during the first months of pregnancy leads, in general, to the death of the conception product and abortion [79].

Maternal infection with parvovirus B19 can start a fetal infection responsible for anemic anasarca [87].

The pregnancy favors the pathogen role of the genital mycoplasmas and bacterial vaginoses [87].

The relation between infection and recurrent abortion

represents an active research subject to establish whether there is a connection between the predisposition inherited during infection and recurrent abortion [92] or there can be proven a strong association between specific groups of vaginal bacteria and the prognosis of pregnancy [93, 64, 33].

The vaginal infections role causing recurrent preterm labor is a research field [94] which seems to be predilectly oriented on bacterial vaginosis, defined as alteration of the vaginal flora, in which the number of lactobacilli, that are normally predominant, is low or the lactobacilli are absent [56, 95].

It was demonstrated on one hand that between 10 and 17 weeks of pregnancy, the vaginal colonization with Ureaplasma urealyticum and/or Bacteroides species, according to some authors and with Trichomonas vaginalis would increase the risk of preterm labor, premature membrane rupture and preterm birth, while between 22 and 28 weeks of pregnancy the vaginal colonization with Gardnerella vaginalis, as in case of Ureaplasma urealyticum also increase the probability of late abortion and preterm birth [96, 97, 98, 99, 94], and, on the other hand, even general infections more or less clinically obvious and even asymptomatic bacteriuria are associated in significant proportions with late abortion and preterm birth [100, 80, 1].

The probable mechanism by means of which the general or local infection contributes to the late abortion (second trimester), preterm labor and membranes rupture [101, 102, 103, 104] is the inflammatory one, triggering a cytokine cascade [105, 56] that once activated it persists and thus, once labor is started, it is too late for antibiotics to be able to delay the evolution of mid trimester abortion or preterm birth, but the antimicrobial therapy, even if tardily applied, can reduce the rate of infectious complications [106, 107, 108, 109, 110, 111, 112, 113].

Nowadays, coming especially from Challis and Mitchell [105], it is more and more accepted the following variant of the relation infection - cytokine cascade- uterine hyperactivity responsible for late abortion and preterm birth: the microorganisms / bacterial endotoxins, most frequently from the vagina and cervix, reaching the extra or intra-amniotic space stimulate the decidual mononuclear phagocytes, triggering the self sustained cytokine cascade, and on the other hand by means of actions on the amniochoriodecidual cells they stimulate directly or indirectly, via the platelet-activating factor and local CRH the release of prostaglandins (PGs) and oxytocin, which, in turn, will stimulate the endometrium in a paracrine manner [114, 115, 116, 117, 76, 118, 119, 120, 121, 122].

Although the circulating levels of the inflammatory cytokines (TNFα, IL-1,6,8 with their agonists and antagonists, including receptors, observable in the serum after 2 hours from the aggression) could prove useful as markers of insulin (infectious, surgical, inflammatory, immune), interpretation of their circulating concentration is sifficult because: a) many cytokines are bioactive at levels well below the threshold of today's traceability; b) release of the cytokines in circulation after the aggression is phasic, not interpretable by means of isolated measurements; c) the cytokines trigger the endogenous mediators cascade, characterized by redundancy, complex and interdependent amplification, and this is why the whole network of hormonal and cytokine mediators with their antagonists has to be evaluated for solid conclusions on the post-traumatic biologic response [123, 124, 125, 126, 127, 128, 129].

Interleukin 6 (IL-6, family of at least 6 phosphoglycoproteins with various abilities) is inhibited by hypercortisolemia and, same as IL-1 and TNFα, but with the difference of the myelostimulant incapacity, is an endogenous pirogen and the primary hepatic inductor of the acute phase proteins synthesis, of which the C-reactive protein (CRP) is the most sensitive one [130, 131].

The C-reactive protein takes its name from its ability to precipitate the C polysaccharide of pneumococcus and its role is that of stimulating the unspecific defense mechanisms of the organism; its concentration increases after 6 hours from the aggression (infectious, tissular lesions), reaching its maximum value after 1 to 3 days from the infectious/mechanical trauma and decreasing 3 days after the inflammatory reaction attenuation, reaction that is defined much more sensibly than fever, leukocytosis and VSH haematids sedimentation speed [132].

The CRP serum dosage (in its most recent variant, using the Tina-quant immunoturbidimetric method, which is as fast and as cheap as counting leucocytes, but without its subjectivism and error factors and moreover without the risks of other, more invasive, evaluations of the infection that impose amniocentesis) is used in monitoring the evolution of bacterial infections, solid cancers and inflammatory diseases [132, 133, 134, 135, 136, 137, 138, 139].

In obstetrics, up to now, it has been proven that on one hand, after a term birth, either vaginal or abdominal, the serum CRP level increases significantly at 24 and respectively 48 hours, decreasing then gradually in the uncomplicated postpartum [140], and on the other, between 27 and 37 gestational weeks, the serum CRP pathological values are significantly correlated with levels over 1500pg/ml of IL-6 from the amniotic fluid [54], while values of the CRP under 2mg/dl indicate the absence of chorioamnionitis for the following 24 hours in 98% of cases with preterm broken membranes [141].

If areas such as the association recurrent abortion – predisposition to infection and/or certain groups of vaginal bacteria are still unclear, the relation infection – second trimester abortion is well established [142] and presents an alarming frequency.

The goal of our prospective and controlled study was the quantitative measurement of serum CRP by means of the immunoturbidimetric technique (Tina-quant) in case of 75 second trimester pregnant women, in order to establish both the evolution of the serum CRP concentration during the second trimester of uncomplicated pregnancy and the

sensibility of the parameter in complicated advanced pregnancies, either with threatened abortion or recurrent abortion of unknown cause or urogenital infections.

## II. Material and Methods

This controlled and prospective study selected 75 non smoking pregnant women, with a gestational age (confirmed in all cases by means of an ultrasound based on the biparietal diameter) of 13 to 27 weeks and who were evaluated, either ambulatory or checked in the Clinic of Obstetrics-Gynecology of the University of Medicine and Pharmacy of Craiova, for normal pregnancy or one with complications, or for checking the postabortum period (with no complications).

After obtaining the consent from all 75 selected pregnant women, in order to supplement the routine evidence with dosing the serum CRP in parallel with counting leucocytes, of a total of 5 ml of venous blood taken in sterile manner, no anticoagulant (4 ml) and respectively with anticoagulant (1 ml), the study subjects were placed in one of the following categories (decided prior to CRP dosing):

a) the group of 18 pregnant women with normal pregnancies, between 13 and 27 weeks, identified during prenatal consultations and whose results in case of dosing the peripheral blood, on one hand served as control values for the group of complicated pregnancies and on the other hand were analyzed in a stratified manner, on pregnancy month, to establish possible correlations between serum levels of CRP and gestational age;

b) the group of subjects to which the CRP dosing and the leukogram were performed both at check-in, either to medically induce (with intravaginal PG – 10 cases) abortion in the second trimester, for fetal reasons such as: retained dead fetus, plurimalformed fetus, broken membranes without uterine contractions on a long cervical canal before 28 weeks or RH isoimmunisation [143], or for threatened abortion of unknown causes (7 cases of second trimester pregnancy in which the diagnose of threatened abortion was established based on the Williams Obstetrics criteria [85]: presence of uterine bleeding +/- pelvic pain, after excluding cervical hemorrhagic lesions, extra uterine pregnancy, gestational trophoblastic disease, low inserted placenta and retroplacental hematoma, on clinic criteria, HCG dosing and echographic criteria) that evolved after 72 hours from check-in with spontaneous expulsion of the conception product, as well as at 12, 24, 36 and 48 hours postabortum uncomplicated (check-out at 72 hours postabortum not identifying a pathology connected to the abortion, reconfirmed ambulatory after the first menstruation);

c) the group of 12 pregnant women in the second trimester with threatened abortion, unknown cause as well, but who didn't evolve towards spontaneous abortion during the hospitalization;

d) the group of 10 pregnant women in the second trimester checked-in because of prior history of at least three consecutive spontaneous abortions (recurrent abortions, according to St. Mary's NHS Trust criteria [142], evaluated after the standard criteria enounced by Rai [144, 145, 146] and Dizon-Townson [147];

e) the group of 18 pregnancies, second trimester, complicated by means of urogenital infections, but without painful uterine contractions and without antibiotic therapy longer than 48 hours in the moment of taking the venous blood for CRP and leukogram (its temperature checked daily).

This final group of pregnant women comprised: 2 cases of spontaneous broken membranes, extremely preterm (at 17 and respectively 19 gestational weeks) of approximately 24 hours and complicated with subclinical chorioamnionitis (confirmed via positive cultures for Escherichia coli, taken in a sterile manner with a transvaginal speculum); 6 cases of subclinical chorioamnionitis with intact membranes, in whose case taking blood for CRP was performed once the presumption diagnosis was established (in the presence of two risk factors for chorioamnionitis such as a dead fetus and oligohydramnios, observed by means of a 2D real-time ultrasound and in the absence of another general or associated to pregnancy pathology, according to Williams Obstetrics [85] but also to the Ornoy group [57]). This presumptive diagnostic was sustained after taking blood for CRP by detecting the appearance of progressive uterine contractions, that culminated with the expulsion of the non macerated fetus at approximately 48 hours from check-in and confirmed by the positive culture for E. coli or Staphylococcus coagulaso-negative from the amniotic liquid taken sterile with a transvaginal speculum, immediately after the spontaneous rupture of the membranes during the abortion; 5 cases of acute pyelonephritis (positive uroculture in 4 cases for E. coli and 1 case of Proteus mirabilis and leukocyte cylinders in the urinary sediment and Giordano positive and fever above 38 degrees Celsius in 3 cases – criteria to diagnose the acute pyelonephritis according to Williams Obstetrics [85]); 5 cases of mixed subclinical vaginitis (trichomoniasis and candidosis) (the examined vaginal smears being positive for Trichomonas and respectively Candida) associated in 4 cases with acute cystitis (diagnose established according to the Williams Obstetrics [85] on: positive uroculture for E. coli in 3 cases and one case of Enterobacter aerogenes and presence of pollakiuria, dysuria and pyuria) and a case of areolar mammary abscess, incised after taking blood for CRP (Staphylococus aureus in the pus culture).

Dosing the serum CRP was performed with the Tina-quant immunoturbidimetric test in a Hitachi 705 automatic analysis system, using both antiCRP specific antibodies as well as positive and negative control serums, following the manufacturer's (Boehringer Mannheim, Austria) instructions, which can be resumed as follows:

*The method principle*: the C-reactive protein forms an immunologic complex with specific antibodies, which determines the increase in turbidity, measured at a wave length ($\lambda$) of 340nm and is directly proportional to the CRP concentration (fig 1).

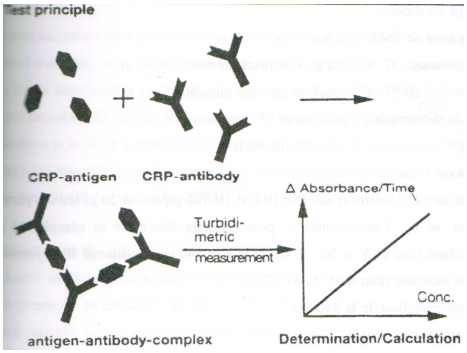


Fig.1

Necessary material: CRP dosing is performed on serum or heparin plasma or treated with EDTA (ethylenediaminetetraacetic acid).

CRP is stable 8 days at +2-8°C or 3 months at -20°C (with the condition to be unfrozen only once).

The control serums used were: Precipath and Precinorm.

Precipath: 3,4 – 5,6 mg/dl

Precinorm: 1,7 – 2,9 mg/dl

In performing the calibration a standard solution with a 7,8mg/dl concentration was used.

Reagents:

1) Buffer solution:

- Tris (hydroxymethyl-aminomethane) 100 mmol/l, pH=7,5
- NaCl 300 mmol/l

2) Anti-CRP buffer (specific antiserum for human CRP):

- Tris (hydroxymethyl-aminomethane) 100 mmol/l, pH=8
- NaCl 300 mmol/l
- polyethylene-glicol 2%

The reagents are stable until the kit's expiration date (written on the box), if they haven't been unsealed. Once used, they are stable 6 weeks at 10°C or 2 weeks at room temperature.

Dosing conditions:

- Wave length: 340nm
- Temperature: 37°C – in automated conditions or 20-25°C – in manual conditions
- Duration of the determination: approximately 15' (automated) or 30' (manual)
- 1 cm vat

Work mode: the HITACHI 705 automated analyzer drops 20µl of serum or plasma, standard and blank. There are performed 3 determinations, and, for each of them there are added 350µl reagent 1 (buffer). Incubation for 5' at 30°C. Reading at λ=340nm. There are added 50µl reagent 2 (antiserum). The second incubation for 5' at 37°C follows.

The absorbances are read at 340nm:

Ax = absorbance of the sample

Ab = absorbance of the blank

As = absorbance of standard

These absorbances are corrected by a blank of distilled water.

The serum CRP concentration of the sample has been calculated using the following formula:

$$Cx = K(Ax-Ab)+Cb$$

where:

C+ – concentration of the sample

Ax – absorbance of the sample

Ab – absorbance of the blank of reagents

Cb – concentration of the reagents blank

K – factor determined with the relation:

$$K=(Cs-Cb)/(As-Ab)$$

where:

Cs – concentration of the standard

As – absorbance of the standard

The HITACHI 705 automated analyzer reads values up to 16mg/dl. For concentrations over this value the sample 1+2 is diluted with a NaCl solution, multiplying the result with 3.

The hemoglobin concentrations under 700mg/dl, bilirubin under 60mg/dl or lipemia (triglycerides) under 6000mg/dl do not interfere in determining the serum CRP.

The sensitivity of the serum CRP dosing Tina-quant test is 0,01mg/dl and the superior limit of the normal in pregnancy has been reported 2mg/dl, being artificially increased with respect to the situations mentioned in the previous paragraph and to the smoker statute of the patient [140, 132, 141].

Microbiological evaluation by smear (vaginal secretion) and cultures (from the amniotic fluid, urine, mammal areolar collection), as well as urine examination and counting leukocytes in the peripheral blood (with the superior limit of the normal in pregnancy, according to the Williams Obstetrics [85], of 12000/ml, unlike the one when not pregnant, of only 10000/ml [148]).

A value of $p<0,05$ represented a statistical significance when comparing results by Student's t and Mann Whitney U tests and respectively analysis of the linear regression, as it dealt with average values or correlations [149].

## III. Results and Discussions

The results of our study, including the demographical characteristics of the studied pregnant women groups are presented in tables I, II and III. The analysis of the linear regression indicates the lack of a positive correlation between serum CRP concentration and gestational age in the second trimester of normal pregnancy after 20 weeks, observation that concords with the literature [140, 141, 54] and allowed us to perform an analysis of the CRP levels based only on affections and not stratified, on age groups, during this investigation.

TABLE I
DEMOGRAPHIC CHARACTERISTICS, CRP AND NO OF SERUM LEUKOCYTES/ML OF THE STUDIED GROUPS PRE AND POST LATE ABORTUM WITHOUT COMPLICATIONS

| Group | A. Second trimester pregnancy with fetal complications that impose | B. Second trimester pregnancy complicated with threatened | A+B. 13-27 weeks pregnancies with non complicated postabortum |
|---|---|---|---|

| | | medical induction of abortion (with intravaginal prostaglandins ) | abortion that evolved after 72 hours from check-in with the spontaneous expulsion of the conception product | evolution, spontaneous or induced |
|---|---|---|---|---|
| Number | | 10 | 7 | 17 |
| Age | | 23,7±1,7 | 26,57±3,53 | 24,88±1,73 |
| Parity | | 1,3±0,66 | 1,7±0,69 | 1,47±0,49 |
| Gestational age (weeks) | | 21,5±1,33 | 22,8±1,67 | 22,05±1,02 |
| CRP mg/dl [no leukocytes / ml] | Preabortum | 1,35±0,36 | 0,72±0,06 | 1,09±0,26 [1156±289] |
| | 24 (12) hours postabortum | *3,31±0,55 | *2,42±0,63 | *3,22±0,38 (2,6±1,06) [8816±533 (8300±828)] |
| | 48 (36) hours postabortum | 2,7±0,52 | 1,55±0,95 | 2,42±0,46 (2,75±0,15) [7660±790 (7033±1256)] |

The mediated results in rows 3 to 8 represent the arithmetic mean ± the standard mean error; * significantly increased ($p<0,05$; Student's t test) values of the serum CRP at 24 hours postabortum with respect to the corresponding preabortum period level.

As in case of the non complicated postpartum [140] our results (table I) constantly indicate a significant (more accentuated after exogenous administration of PG in induced abortion) and maximum increase of serum CRP concentration (but not of the leukocytes number) at 24 hours postabortum, followed by a progressive decrease, of minimum 18%, of the same parameter, at 48 hours after non complicated second trimester abortion.

Recognizing the constancy of such an evolution curve of the late non complicated postabortum serum CRP level presents a diagnostic significance for the complicated cases, for instance infectious.

Stimulating the serum CRP by exogenous administration of PG explains, at least partially, the significant growth of CRP postabortum and concords with prior observations on this relation, both postpartum [140] as well as outside of the pregnancy [105, 56].

The table II shows that only the pregnancy complicated with urogenital bacterial infections is accompanied by pathological values of the serum CRP (but not of the leukogram) that are significantly increased with respect to those from a normal pregnancy or one complicated with threatened abortion of unknown and non evolutive cause, or recurrent abortions in history.

TABLE II
DEMOGRAPHIC CHARACTERISTICS, CRP AND NUMBER OF LEUKOCYTES/ML OF THE SECOND TRIMESTER OF NORMAL AND COMPLICATED PREGNANCY STUDIED GROUPS

| Group | Normal pregnancy | Threatened abortion | Pregnancy after at least 3 consecutive spontaneous abortions in history (recurrent abortions) | Pregnancy complicated by urogenital infections |
|---|---|---|---|---|
| Number | 18 | 12 | 10 | 18 |
| Age | 21,7±0,91 | 23±0,96 | 25±0,09 | 23,88±1,52 |
| Parity | 0,33±0,13 | 0,41±0,19 | 0,1±0,1 | 0,72±0,39 |
| Gestational age (weeks) | 21,6±1,07 | 15,08±2,3 | 18,2±1,34 | 20,2±0,86 |
| CRP: mg/dl | 0,94±0,06 | 1,22±0,17 | 1,35±0,36 | *3,83±0,65 |
| No of leukocytes / ml (limits) | 7156±289 (5400-9800) | 6754±352 (4200-8500) | 6960±410 (5000-8500) | 7572±403 (4600-10400) |

The mediated results in rows 3 to 8 represent the arithmetic mean ± the standard mean error; * significantly increased ($p<0,05$) value of CRP with respect to the corresponding result in case of a normal pregnancy (Student's t test) and complicated (Mann Whitney U test) with threatened abortion and recurrent abortions in history.

From table III it results that 72% of the second trimester pregnancies complicated with urogenital infections (including infections of the inferior urogenital tract as well as mammary abscess) have been identified by means of pathological values of the serum CRP (concluding that CRP sensitivity is 72% compared to 16% for the thermal curve and 0% for leukocytosis), while 100% of the pregnant women with serum CRP concentration > 2mg/dl have been diagnosed with subclinical chorioamnionitis or acute pyelonephritis (100% positive predictive value).

The fact that the serum CRP in case of second trimester pregnant women increases pathologically only in case of recent high urinary bacterial infections and moreover this increase is accompanied just by thermal ascension of various intensities and not by hyperleukocytosis, on one hand sustains the common embrionary origin of the high urogenital segments with similar reactivity to aggressions, and on the other hand suggests as primary growth hepatic inductor for serum CRP the members of the IL-6 family and not IL-1b or TNF-α, that are strong myelostimulants [130, 128, 129], thus in consensus with the positive correlation described from 27 to 37 weeks of pregnancy between the pathological level of serum CRP and that of IL-6 from the amniotic fluid infected in conditions of

intact fetal membranes [54].

TABLE III
CRP SENSITIVITY AND NUMBER OF SERUM LEUKOCYTES/ML AS WELL AS FEVER IN THE SECOND TRIMESTER OF PREGNANCY COMPLICATED WITH UROGENITAL INFECTIONS

| Group | Subclinical chorioamnionitis with spontaneously broken membranes | Subclinical chorioamnionitis with intact membranes | Acute pyelonephritis | Candidosic and trichonomiasic subclinical vaginitis associated with acute cystitis or areolar mammary abscess | CRP/ no of leukocytes/ fever sensitivity (%) |
|---|---|---|---|---|---|
| Number | 2 | 6 | 5 | 5 | 18 |
| Age | 37 (36-38) | 22,5 (15-32) | 24 (21-28) | 20,2 (16-22) | - |
| Parity | 4,5 (2-7) | 0,16 (0-1) | 0 | 0,6 (0-1) | - |
| Gestational age (weeks) | 18 (17-19) | 19,5 (14-25) | 23,2 (20-27) | 19 (15-23) | - |
| CRP: mg/dl | 6,65 (4,4-8,9) | 3,15 (2,5-4,1) | 6,4 (2,9-9,5) | 0,98 (0,4-1,9) | 13/18 (72%) |
| No of leukocytes / ml (limits) | 8700 (8400-9000) | 6190 (5600-10000) | 8480 (6000-10400) | 6720 (4600-8200) | 0/18 (0%) |
| No of case of fever (>38°C) | 0 | 0 | 3 | 0 | 3/18 (16%) |

The results from rows 2 to 6 represent the mean values (limits).

But, comparing the observations on the serum C-reactive protein (CRP) in second trimester of pregnancy complicated with recurrent abortion and threatened abortion from this study and the results obtained by us [150, 151] by means of flux cytometry to sketch the peripheral immune profile of the pregnancy complicated with the same morbid entities, it results that the most frequent recurrent abortion mechanism, within the population studied here, is expressed by a cytokine cascade variant other than that involving interleukin 6 (the main serum CRP inductor).

In case of the pregnant women in second trimester with low urogenital infectious complications, or due to urealyticum Ureaplasma (associated to some cases of recurrent abortion, [91]), or with threatened abortion of unknown cause, not evolving immediately, only the simultaneous study of serum CRP with intraamniotic IL-6 (alongside the bacteriology via the reaction of the polymerase chain, [152]) and cortisolemia, in the conditions of objectivation and infection with Ureaplasma urealyticum by cervical cultures, could define to what extent the lack of pathological growth of serum CRP observed by us in tables II and III for these morbid entities is due to the inhibition of CRP growth by the simultaneous hypercortisolemia [131] associated to these affections or to the absence/insufficiency of IL-6 from the cytokine cascade induced either by the minor trauma associated to threatened abortion of unknown cause and non evolving, or by Ureaplasma urealyticum or by the vaginal trichomoniasis and candidosis and/or inherent to the different embrionary origin of the inferior urogenital tract with respect to the superior one.

## IV. CONCLUSIONS

1) Experimental studies, like those mentioned previously but numerically extended, would be necessary to define in a clearer manner the limits of the current investigation, but also the possible use of serum CRP dosing as predictive marker of extremely preterm labor due to inflammatory cause, for which the tocolysis presents encouraging perspectives by means of IL-10 type agents.
2) The obtained results suggest that the dynamic measurement of the serum CRP concentration could prove to be a valuable and practical predictive marker of intrauterine infection, both in the late periabortum period as well as in the latency phase of the extremely premature rupture of the membranes or even when the fetal membranes are intact in the second trimester.
3) In this latter situation (intact fetal membranes in the second gestational trimester), given the observation in our study of the correlation between serum CRP and positive culture of the amniotic fluid, the less invasive serum CRP test could become a valuable screening for intrauterine infection on intact membranes in the middle trimester, to precede the amniocentesis or even replace it in case of coexistence of two other risk factors of chorioamnionitis and in the absence of painful uterine contractions, that would allow the application of etiopathogenic therapy.

## REFERENCES

[1] MJNC Keirse, "New perspectives for effective treatment of preterm labor", *Am J Obstet Gynecol*, 173:618, 1995
[2] M. Delcroix, C. Gomez, "Urgences obstetricales", *Soins en Gynecologie Obstetrique*, Paris, Ed. Maloine, 270-273, 2005
[3] J.A. McGregor, J.I. French, "Evaluation of preterm birth misleading", *Am J Obstet Gynecol*, 174: 1392, 1996.
[4] P.J. Meis, R. Michielutte, T.J. Peters, B.H. Wells, E.R. Sands, E.C. Coles, K.A. Johns, "Factors associated with preterm birth in Cardiff, Wales. I Univariable and multivariable analysis", *Am J Obstet Gynecol*, 173: 590, 1995
[5] P.J. Meis, R. Michielutte, T.J. Peters, B.H. Wells, E.R. Sands, E.C. Coles, K.A. Johns, "Factors associated with preterm birth in Cardiff, Wales. II Indicated and spontaneous preterm birth", *Am J Obstet Gynecol*, 173: 597, 1995
[6] Y. Chavkin, C. Kupfersztain, P. Guedj, M. Stark, "Conservative treatment with successful outcome of a triplet pregnancy after the miscarriage of one fetus in the second trimester", *Int J Gynecol Obstet*, 44: 161, 1994.
[7] K. Ellett, E.J. Buxton, D.M. Luesley, "The effect of ethnic origin on the rate of spontaneous late mid-trimester abortion", *Ethn Dis*, 2: 84, 1992

[8] R.L. Copper, RL. Goldenberg, A. Das, N. Elder, M. Swain, G. Norman, R. Ramsey, P. Cotroneo, B.A. Collins, F. Johnson, P. Jones, P. Meier and the National Institute of Child Health and Human Development Maternal - Fetal Medicine Units Network., "The preterm prediction study: Maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation", *Am J Obstet Gynecol*, 175: 1286, 1996

[9] R.L. Goldenberg, J.D. Iams, M. Miodovnik, J.P. Van Dorsten, G. Thurnau, S. Bottoms, B.M. Mercer, P.J. Meis, A.H. Moawad, A. Das, S.N. Caritis, D. McNellis and the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network, "The preterm prediction study: Risk factors in twin gestations", *Am J Obstet Gynecol*, 175: 1047, 1996a.

[10] G.C. Windham, "Caffeine not linked to miscarriage, but alcohol is", *Fertility weekly*, 22: 1, 1997.

[11] R.M. Pickering, J.J. Deeks, "Risks of delivery during the 20th to the 36th week of gestation", *Int J Epidemiol*, 20: 456, 1991.

[12] B.M. Mercer, R.L. Goldenberg, A. Das, A.H. Moawad, J.D. Jams, P.J. Meis, R.L. Copper, F. Johnson, E. Thom, D. McNellis, M. Miodovnik, M.K. Menard, S.N. Caritis, G.R. Thurnan, S.F. Bottoms, J. Roberts, "The preterm prediction study: A clinical risk assessment system", *Am J Obstet Gynecol*, 174: 1885, 1996.

[13] P.C. Greig, W.N.P. Herbert, B.L. Robinette, L.A. Teot, "Amniotic fluid interleukin - 10 concentrations increase throughout pregnancy and are elevated in patients with preterm labor associated with intrauterine infection", *Am J Obstet Gynecol*, 173: 1223, 1995.

[14] S.J. Fortunato, M. Ramkumar, K.F. Swan, S.J. Lombardi, "Interleukin-10 inhibition of interleukin-6 in human amniochorionic membrane: Transcriptional regulation", *Am J Obstet Gynecol*, 175: 1057, 1996b.

[15] S.J. Fortunato, R. Menon, S.J. Lombardi, "Interleukin - 10 and transforming growth factor-β inhibit amniochorion tumor necrosis factor - α production by contrasting mechanisms or action: therapeutic implications in prematurity", *Am J Obstet Gynecol*, 177: 803, 1997.

[16] A.O. Rust, J.A. Bofill, R.M. Arriola, M.E. Andrew, J.C. Morrison, "The clinical efficacy of oral tocolytic therapy", *Am J Obstet Gynecol*, 175: 838, 1996.

[17] H.A. Akouri, S.E. White, J. Homan, V.Y.T. Cheung, B.S. Richardson, A.D. Bocking, "Failure of magnesium sulphate infusion to inhibit uterine activity in pregnant sheep", *Am J Obstet Gynecol*, 177: 185, 1997.

[18] L.M. Casey, C.E.L. Brown, M. Peters, P.C. Mac Donald, "Endothelin levels in human amniotic fluid at mid-trimester and at term before and during spontaneous labor", *J Clin Endocrinol Metab*, 76 (6): 1647, 1993.

[19] G.J. Valenzuela, C.W. Hewitt, C.A. Ducsay, "Endothelin - 1 potentiates the in vitro contractile response of pregnant human myometrium to oxytocin", *Am J Obstet Gynecol*, 172: 1573, 1995.

[20] G. Rizzo, A. Capponi, D. Rinaldo, D. Tedeschi, D. Arduini, C. Romanini, "Interleukin - 6 concentrations in cervical secretions identify microbial invasion of the amniotic cavity in patients with preterm labor and intact membranes", *Am J Obstet Gynecol*, 175: 812, 1996a.

[21] K.D. Wenstrom, W.W. Andrews, T. Tsunenbu, M.B. Du Bard, K.E. Johnston, P. Hemstreet, "Elevated amniotic fluid interleukin -6 levels at genetic amniocentesis predict subsequent pregnancy loss", *Am J Obstet Gynecol*, 175: 830, 1996.

[22] A.P. Murtha, G.C. Phillip, C.E. Jimmerson, B.R. Johnson, J. Allen, W.N.P. Herbert, "Maternal serum interleukin - 6 concentrations in patients with preterm premature rupture of membranes and evidence of infection", *Am J Obstet Gynecol*, 175: 966, 1997.

[23] B.H. Yoon, R. Romero, S.H. Yang, J.K. Yun, J.O. Kim, J.H. Choi, H.C. Syn, "Interleukin - 6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia", *Am J Obstet Gynecol*, 174: 1433, 1996.

[24] R.L. Goldenberg, T. Tamura, M. Du Bard, K.E. Johnston, R.L. Copper, Y. Neggers, "Plasma ferritin and pregnancy outcome", *Am J Obstet Gynecol*, 175: 1356, 1996b.

[25] E. Leikin, D. Garry, P. Visintainer, U. Verma, N. Tejani, "Correlation of neonatal nucleated red blood cell counts in preterm infants with histologic chorioamnionitis", *Am J Obstet Gynecol*, 177: 27, 1997.

[26] J.E. Sampson, R.P. Theve, R.N. Blatman, T.D. Shipp, D.W. Bianchi, B.E. Ward, R.M. Jack, "Fetal origin of amniotic fluid polymorphonuclear leukocytes", *Am J Obstet Gynecol*, 176: 77, 1997.

[27] A.E. Reece, "Pregnancy outcome: influence of antiphospholipid antibody titer, prior pregnancy losses and treatment", *J Reprod Med*, 42: 49, 1997.

[28] R.E. Mayer, S.J. Thompson, C.L. Addy, C.Z. Garrison, R.G. Best, "Maternal serum placental alkaline phosphatase level and risk for preterm delivery", *Am J Obstet Gynecol*, 173: 181, 1995.

[29] M.J. Kupferminc, A.M. Peaceman, D. Aderka, D. Wallach, R.M. Peyser, J.B. Lessing, M.L. Socol, "Soluble tumor necrosis factor receptors in maternal plasma and second trimester amniotic fluid", *Am J Obstet Gynecol*, 173: 900, 1995.

[30] V.Z. Gordina, "Serum oxytocin and oxytocinase activities in women during pregnancy", *Akush Ginecol Mosk*, 4: 11, 1991.

[31] P. Borella, A. Szilagyi, G. Than, I. Csaba, A. Giardino, F. Facchinetti, "Maternal plasma concentrations of magnesium, calcium, zinc and copper in normal and pathological pregnancies", *Sci Total Environ*, 99: 67, 1990.

[32] A. Kurjak, I. Zalud, M. Predanic, S. Kupesic, "Transvaginal color and pulsed Doppler study of uterine blood flow in the first and early second trimesters of pregnancy: normal versus abnormal", *J Ultrasound Med*, 13: 43, 1994.

[33] C.Y. Spong, A. Ghidini, D. Sherer, J.C. Pezzullo, M. Ossandon, G.S. Eglinton, "Angiogenin: A marker for preterm delivery in midtrimester amniotic fluid", *Am J Obstet Gynecol*, 176: 415, 1997.

[34] D.M. Svinarich, N.A. Wolf, R. Gomez, B. Gonik, R. Romero, "Detection of human defensin 5 in reproductive tissues", *Am J Obstet Gynecol*, 176: 470, 1997.

[35] F. Facchinetti, G. Garuti, F. Petraglia, F. Mercantini, A.R. Genazzani, "Changes in beta-endorphin in fetal membranes and placenta in normal and pathological pregnancies", *Acta Obstet Gynecol Scand*, 69: 603, 1990.

[36] F. Petraglia, S. Tabanelli, M.C. Galassi, G.C. Garuti, A.C. Mancini, A.R. Genazzani, E. Gurpide, "Human decidua and in vitro decidualized endometrial stromal cells at term contain immunoreactive corticotropin - releasing factor (CRF) and CRF messenger ribonucleic acid", *J Clin Endocrinol Metab*, 74: 1427, 1992.

[37] G.S. Berkowitz, R.H. Lapinski, C.J. Lockwood, P. Florio, C.B. Prince, F. Petraglia, "Corticotropin - releasing factor and its binding protein: Maternal serum levels in term and preterm deliveries", *Am J Obstet Gynecol*, 174: 1477, 1996.

[38] K.A. Sorem, C.B. Smikle, D.K. Spencer, B.A. Yoder, M.A. Graveson, T.M. Silver-Khodr, "Circulating maternal corticotropin releasing hormone and gonadotropin - releasing hormone in normal and abnormal pregnancies", *Am J Obstet Gynecol*, 175: 912, 1996.

[39] B.H. Yoon, J.K. Jun, R. Romero, K.H. Park, R. Gomez, J.H. Choi, J.O. Kim, "Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1β and tumor necrosis factor - α), neonatal brain white matter lesions and cerebral palsy", *Am J Obstet Gynecol*, 177: 19, 1997.

[40] D. Garry, R. Gigueroa, M.A. Rosenfeld, E. Martinez, P. Visintainer, N. Tejani, "A comparison of rapid amniotic fluid markers in the prediction of microbial invasion of the uterine cavity and preterm delivery", *Am J Obstet Gynecol*, 175: 1336, 1996.

[41] J.W. Kasik, E.J. Rice, "An increase in expression of the lipocalin 24p3 is found in mouse uterus coincident with birth", *Am J Obstet Gynecol*, 173: 613, 1995.

[42] J.D. Iams, R.L. Goldenberg, P.J. Meis, B.M. Mercer, A. Moawad, A. Das, E. Thom, D. McNellis, R.L. Cooper, F. Johnson, J.M. Roberts, "The length of the cervix and the risk of spontaneous premature delivery", *N Engl J Med*, 334: 567, 1996.

[43] M. Bloechle, H. Halle, R. Bollmann, "Vaginal ultrasound cervix imaging in pregnancy - predictive value of suspicious findings on the subsequent course of pregnancy", *Zentralbl Gynekol*, 116: 697, 1994.

[44] G. Blanch, K.S.J. Olah, S. Walkinshaw, "The presence of fetal fibronectin in the cervicovaginal secretions of women at term - Its role in the assessment of women before labor induction and in the investigation of the physiologic mechanisms of labor", *Am J Obstet Gynecol*, 174: 262, 1995.

[45] R.F. Feinberg, H.J. Kliman, V. Bedian, F. Monzon-Bordonaba, A.W. Menzin, C.L. Wang, "Monoclonal antibody X18A4 identifies an oncofetal fibronectin epitope distinct from the FDC-6 binding site", *Am J Obstet Gynecol*, 172: 1526, 1995.

[46] J.D. Iams, D. Casal, J.A. McGregor, M.T. Goodwin, U.S. Creaden, R. Lowensohn, G. Lockich, "Fetal fibronectin improves the accuracy of diagnosis of preterm labor", *Am J Obstet Gynecol*, 173: 141, 1995.
[47] J. Bartnicki, D. Casal, U.S. Kireaden, E. Saling, K. Vetter, "Fetal fibronectin in vaginal specimens predicts preterm delivery and very low birth weight infants", *Am J Obstet Gynecol*, 174: 971, 1996.
[48] R.E. Bittar, A.A. Yamasaki, S. Sasaki, M. Zugaib, "Cervical fetal fibronectin in patients at increased risk for preterm delivery", *Am J Obstet Gynecol*, 175: 178, 1996.
[49] J.B. Greenhagen, J. Van Wagoner, D. Dudley, C. Hunter, M. Mitchell, V. Logsdon, D. Casal, M. Varner, "Value of fetal fibronectin as a predictor of preterm delivery for a low-risk population", *Am J Obstet Ginecol*, 175: 1054, 1996.
[50] G. Rizzo, A. Capponi, D. Arduini, C. Lorido, C. Romanini, "The value of fetal fibronectin in cervical and vaginal secretions and of ultrasonografic examination of the uterine cervix in predicting premature delivery for patients with preterm labor and intact membranes", *Am J Obstet Gynecol*, 175: 1146, 1996b.
[51] R.L. Goldenberg, B.M. Mercer, J.D. Iams, A.H. Moawad, P.J. Meis, A. Das, D. McNellis, M. Miodovnik, K. Menard, S.N. Caritis, G. Thurnau, S.F. Bottoms and the National Institute of Child Health and Human Development Maternal - Fetal Medicine Units Network., "The preterm prediction study: Patterns of cervicovaginal fetal fibronectin as predictors of spontaneous preterm delivery", *Am J Obstet Gynecol*, 177: 8, 1997.
[52] A.M. Peaceman, W.W. Andrews, J.M. Thorp, S.P. Cliver, A. Lukes, J.D. Iams, L. Coultrip, N. Eriksen, H. Holbrook, J. Elliott, C. Ingardia, M. Pietrantoni, "Fetal fibronectin as a predictor of preterm birth in patients with symptoms: A multicenter trial", *Am J Obstet Gynecol*, 177: 13, 1997.
[53] P. Rozenberg, F. Goffinet, L. Malaguida, Y. Guidicelli, M. Perdu, I. Houssin, I. Sage-Femme Nisand, "Evaluating the risk of preterm delivery: A comparison of fetal fibronectin and transvaginal ultrasonographic measurement of cervical length", *Am J Obstet Ginecol*, 176: 196, 1997.
[54] D.R. Burrus, J.M. Ernest, J.C. Veille, "Fetal fibronectin, interleukin - 6 and C - reactive protein are useful in establishing prognostic subcategories of idiopathic preterm labor", *Am J Obstet Gynecol*, 173: 1258, 1995.
[55] D.A. Gaillard, P. Paradis, A.V. Lallemand, V.M. Vernet, J.S. Carquin, C.G. Chippaux, B.J. Visseaux - Coletto, "Spontaneous abortions during the second trimester of gestation", *Arch Pathol Lab Med*, 117: 1022, 1993.
[56] M.J.C.N. Keirse, H. McDonald, "Infection and preterm labor", *Progress in preterm*, 1(2): 5, 1996.
[57] A. Ornoy, K. Crone, G. Altshuler, "Pathological features of the placenta in fetal death", *Arch Pathol Lab Med*, 100: 367, 1976.
[58] I. Goldstein, E.Z. Zimmer, D. Merzbach, B.A. Peretz, E. Paldi, "Intraamniotic infection in the very early phase of the second trimester", *Am J Obstet Gynecol*, 163: 1261, 1990.
[59] E. Saling, S. Brandt-Niebelschutz, C. Schmitz, "Prevention of late abortion and high risk premature labor - measures suited for routine practice", *Z Geburtshilfe Perinatol*, 195: 209, 1991.
[60] R.H. Rudbeck, U. Henriques, "Fetal and perinatal infections. A consecutive study", *Pathol Res Pract*, 188: 135, 1992.
[61] R. Romero, R. Gonzales, W. Sepulveda, F. Brandt, M. Ramirez, Y. Sorokin, M. Mazor, M.C. Treadwell, D.B. Cotton, "Infection and labor. VIII. Microbial invasion of the amniotic cavity in patients with suspected cervical incompetence: prevalence and clinical significance", *Am J Obstet Gynecol*, 167: 1086, 1992b.
[62] H. Fernandez, B. Montuclard, M. Guilbert, "Does intraamniotic infection in the early phase of the second trimester really exist?", *Am J Obstet Gynecol*, 175: 1077, 1996.
[63] D.E. Soper, C.G. Mayhall, J.W. Froggatt, "Characterization and control of intraamniotic infection in an urban teaching hospital", *Am J Obstet Gynecol*, 175: 304, 1996.
[64] F. Arias, L. Rodrigues, L. Rayne, F.T. Kraus, "Maternal placental vasculopathy and infection: Two distinct subgroups among patients with preterm labor and preterm ruptured membranes", *Am J Obstet Gynecol*, 168: 585, 1993.
[65] C. Bulucea, N. Mastorakis, M. Paun, R. Marcu, "Histopathological Placental Screening as Valuable and Non-Invasive Method for Assessing Etiology of Second Trimester Recurrent Abortion, Recent Advances in Clinical Medicine", *Proceedings of the International Conference on Medical Histology and Embryology* (HISTEM '10), 180:349, Cambridge, 2010
[66] C.A. Bulucea, N.E. Mastorakis, M.F. Paun, A.D. Neatu, A.G. Neatu, "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", *WSEAS Transactions on Biology and Biomedicine*, Issue 3, Volume 7, 146-157, July 2010
[67] D.A. Schwartz, B. Walker, B. Furlong, E. Breding, A. Someren, "Cytomegalovirus in a macerated second trimester fetus: persistent viral inclusions on light and electron microscopy", *South Med J*, 83: 1357, 1990.
[68] E.P. Fedotova, G.V. Shastina, "Intrauterine mycoplasmosis in late miscarriage", *Arkh Patol*, 56: 61, 1994.
[69] C.A. Labarrere, S.D. Carson, W.P. Faulk, "Tissue factor in chronic villitis of unestablished etiology", *J Reprod Immunol*, 19: 225, 1991.
[70] C.M. Salafia, H.E. Mangam, C.A. Weigl, G.J. Foye, L. Silberman, "Abnormal fetal heart rate patterns and placental inflammation", *Am J Obstet Gynecol*, 160: 140, 1989.
[71] P. Puggina, F. D'Amato, A. Spallone, P. Mignano, "Placental chorioangioma, Report of a case in relation to authors' experience", *Minerva Ginecol*, 43: 61, 1991.
[72] L.C. Horn, M. Rosenkranz, K. Bilek, "The value of placental histology for the detection of genetically - induced abortions", *Z Geburtshilfe Perinatol*, 195: 47, 1991.
[73] S.A. Stepanov, A.K. Kirichenko, I.A. Aleskeev, "Chronic placental insufficiency and histogenesis of fetal thyroid in late spontaneous abortions", *Arkh Patol*, 55: 64, 1993.
[74] A.P. Milovanov, E.G. Kurik, "Morphometry of the non-villous placental cytotrophoblast in the interrupted pregnancy", *Arkh Patol*, 52: 26, 1990.
[75] A.L. Bernal, D.J. Hansell, T.Y. Khong, J.W. Keeling, A.C. Turnbull, "Prostaglandin E production by the fetal membranes in unexplained preterm labor and preterm labor associated with chorioamnionitis", *Br J Obstet Gynecol*, 96: 1133, 1989.
[76] C.W. van der Elst, A.L. Bernal, C.C. Sinclair-Smith, "The role of chorioamnionitis and prostaglandins in preterm labor", *Obstet - Gynecol*, 77: 672, 1991.
[77] C. Bulucea, N. Mastorakis, M. Paun, A. Neatu, "Evaluating the infection in the second trimester of pregnancy by C-reactive protein dosing", North Atlantic University Union Proceedings of the World Medical Conference, 149-155, Malta, 2010
[78] R. Durieux, A.J. Bret, "Pseudomonas aeruginosa et l'avortement spontané. Saprophytisme. Parasitisme. Patogenie", *Rev fr Gynécol Obstét*, 62 (9): 451, 1967.
[79] R. Vokaer, *Traité d'Obstétrique*, Masson, Les Presses de l'Université Laval, Québec, 1985.
[80] O.V. Akimov, "Pulmonary echinococcosis, hepatic opisthorchiasis and generalized trichinelliasis combined with pregnancy", *Arkh Patol*, 55: 81, 1993.
[81] J. Cazenave, "Le diagnostic anténatal de la toxoplasmose congénitale", *Références en Gynécologie Obstétrique*, 1: 1, 1993.
[82] A. Lindstrand, S. Bergstrom, A. Bugalho, G. Zanconato, A.M. Helgesson, B. Hederstedt, "Prevalence of syphilis infection in Mozambican women with second trimester miscarriage and women attending antenatal care in second trimester", *Genitourin Med*, 69: 431, 1993.
[83] C. Scieux, A. Blanchi, N. Branat, S. Henry, "Le diagnostic biologique des infections urogénitales à Chlamydia trachomatis", *Références en Gynécologie Obstétrique*, 1: 3, 1993.
[84] D. Desprez, P. Blanc, D. Larrey, G. Galindo, J. Ramos, G. Feldmann, H. Michel, "Acute hepatitis caused by Listeria monocytogenes infection", *Gastroenterol Clin Biol*, 18: 516, 1994.
[85] *Williams Obstetrics*, 19th Edition, Prentice-Hall International Inc, 1993.
[86] I. Munteanu, *Avortul*, Tratat de obstetrica, editia II, vol 2, Bucuresti, Editura Academiei Romane, 815-848, 2006
[87] M. Delcroix, C. Gomez, "Fievre et grossesse, Infections et grossesse", *Soins en Gynecologie Obstetrique*, Paris, Ed. Maloine, 311, 2005
[88] H. Marret, J. Wagner-Ballon, H. Guyot, "Elle est enceinte et a la rubéole", 101 questions en gynécologie – obstétrique, Paris, Ed. Masson, 28-33, 2004

[89] H. Marret, J. Wagner-Ballon, H. Guyot, "Elle est enceinte et a la varicelle", 101 questions en gynécologie – obstétrique, Paris, Ed. Masson, 33-37, 2004
[90] R. Merger, *Précis d'Obstétrique*, Masson, 1989.
[91] P.A. Quinn, M. Petric, M. Barkin, J. Butany, C. Derzko, M. Gysler, K.I. Lie, A.B. Shewchuck, J. Shuber, E. Ryan, M.L. Chipman, "Prevalence of antibody to Chlamydia trachomatis in spontaneous abortion and infertility", *Am J Obstet Gynecol*, 156: 291, 1987.
[92] D.N. Burns, P. Nourjah, H. Minkoff, J. Korelitz, R.J. Biggar, S. Landesman, A. Rubinstein, D. Wright, R.P. Nugent, "Changes in CD4+ and CD8+ cell levels during pregnancy and postpartum in women seropositive and seronegative for human immunodeficiency virus-1", *Am J Obstet Gynecol*, 174: 1461, 1996.
[93] R. Romero, C.M. Salafia, A.P. Athanassiadis, S. Hanaoka, M. Mazor, W. Sepulveda, M.B. Braken, "The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology", *Am J Obstet Gynecol*, 166: 1382, 1992a.
[94] E.R. Newton, J. Piper, W. Peairs, "Bacterial vaginosis and intraamniotic infection", *Am J Obstet Gynecol*, 176: 672, 1997.
[95] M. Delcroix, C. Gomez, "Infections gynécologiques", *Soins en Gynécologie Obstétrique*, Paris, Ed. Maloine, 79-93, 2005
[96] P.E. Hay, R.F. Lamont, D. Taylor Robinson, D.J. Morgan, C. Ison, J. Pearson, "Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage", *Br Med J*, 308: 295, 1994.
[97] J.M. Llahi Camp, R. Rai, C. Ison, L. Regan, D. Taylor Robinson, "Association of bacterial vaginosis with a history of second trimester miscarriage", *Hum Reprod*, 11: 1575, 1996.
[98] S. Wood, "Preterm delivery and bacterial vaginosis - Logistic regression analysis", *Am J Obstet Gynecol*, 175: 753, 1996.
[99] J.A. Regan, M.A. Kebanoff, R.P. Nugent, D.A. Eschenbach, W.C. Blackwelder, Y. Lou, R.S. Gibbs, P.J. Rettig, D.H. Martin, R. Edelman, "Colonization with group B streptococci in pregnancy and adverse outcome", *Am J Obstet Gynecol*, 174: 1354, 1996.
[100] D.G. Hesse, K.J. Tracey, Y. Fong, K.R. Manogue, M.A. Palladino, A. Cerami, T. Shires, S.F. Lowry, "Cytokine appearance in human endotoxemia and primate bacteremia", *Surg Gynecol Obstet*, 166: 147, 1988.
[101] M. Cooperstock, "Mechanisms of preterm premature rupture of membranes", *Am J Obstet Gynecol*, 174: 1672, 1996.
[102] F. Vadillo – Ortega, A. Hernandez, G. Gonzalez – Avila, L. Bermejo, K. Iwata, J.F. Strauss, "Increased matrix metalloproteinase activity and reduced tissue inhibitor of metalloproteinases -1 levels in amniotic fluids from pregnancies complicated by premature rupture of membranes", *Am J Obstet Gynecol*, 174: 1371, 1996.
[103] P.L. Collins, J.J. Moore, E. Idriss, Grad. Biomed. Eng. and T.M. Kulp, "Human fetal membranes inhibit calcium L - channel activated uterine contractions", *Am J Obstet Gynecol*, 175: 1173, 1996.
[104] H.W. Kilbride, J. Yeast, D.W. Thibeault, "Defining limits of survival: Lethal pulmonary hypoplasia after midtrimester premature rupture of membranes", *Am J Obstet Gynecol*, 175: 675, 1996.
[105] J.R.G. Challis, M.D. Mitchell, "Basic mechanisms of preterm labor", *Research and Clinical Forums*, 16: 39, 1994.
[106] E. Saling, "Effective measures for the prevention of late abortions and premature births", *Early Hum Dev*, 29: 15, 1992.
[107] J.A. McGregor, J.I. French, R. Parker, D. Draper, E. Patterson, J. Ward, K. Thorsgard, J. McFee, "Prevention of premature birth by screening and treatment for common genital tract infections: Results of a prospective controlled evaluation", *Am J Obstet Gynecol*, 173: 157, 1995.
[108] M. Gordon, P. Samuels, P. Shubert, F. Johnson, C. Gebauer, J. Iams, "A randomized, prospective study of adjunctive ceftizoxime in preterm labor", *Am J Obstet Gynecol*, 172: 1546, 1995.
[109] S.M. Cox, V.R. Bohman, L.M. Sherman, K.J. Leveno, "Randomized investigation of antimicrobials for the prevention of preterm birth", *Am J Obstet Gynecol*, 174: 206, 1996.
[110] J.A. Grable, P.M. Garcia, D. Perry, M.L. Socol, "Group B Streptococcus and preterm premature rupture of membranes: A randomized double - blind clinical trial of antepartum ampicillin", *Am J Obstet Gynecol*, 175: 1036, 1996.
[111] B.M. Mercer, "Antibiotic treatment for preterm premature rupture of membranes", *Am J Obstet Gynecol*, 175: 756, 1996.
[112] S.M. Lovett, J.D. Weiss, M.J. Diogo, P.T. Williams, J.T. Garite, "A prospective double-blind, randomized, controlled clinical trial of ampicillin-sulbactam for preterm premature rupture of membranes in women receiving antenatal corticosteroid therapy", *Am J Obstet Gynecol*, 176: 1030, 1997.
[113] R.S. Gibbs, D.A. Eschenbach, "Use of antibiotics to prevent preterm labor", *Am J Obstet Gynecol*, 177: 375, 1997.
[114] K. Anwer, M. Monga, B.M. Sanborn, "Epidermal growth factor increases phosphoinositide turnover and intracellular free calcium in an immortalized human myometrial cell line independent of the arachidonic acid metabolic pathway", *Am J Obstet Gynecol*, 174: 676, 1996.
[115] J.K. Pollard, M.D. Mitchell, "Intrauterine infection and the effects of inflammatory mediators on prostaglandin production by myometrial cells from pregnant women", *Am J Obstet Gynecol*, 174: 682, 1996.
[116] E.E. Maradny, N. Kanayama, A. Halim, K. Maehara, T. Terao, "Stretching of fetal membranes increases the concentration of interleukin - 8 and collagenase activity", *Am J Obstet Gynecol*, 174: 843, 1996.
[117] D.A. Calhoun, C. Rosa, R.D. Christensen, "Transplacental passage of recombinant human granulocyte colony - stimulating factor in women with an imminent preterm delivery", *Am J Obstet Gynecol*, 174: 1306, 1996.
[118] S.J. Fortunato, R.P. Menon, K.F. Swan, R. Menon, "Inflammatory cytokine (interleukins 1, 6 and 8 and tumor necrosis factor - α) release from cultured human fetal membranes in response to endotoxic lipopolysaccharide mirrors amniotic fluid concentrations", *Am J Obstet Gynecol*, 174: 1885, 1996a.
[119] D.M. Svinarich, O.M. Bitonti, R. Romero, B. Gonik, "Induction and postranslational expression of cytokines in a first trimester trophoblast cell line by lipopolysaccharide", *Am J Obstet Gynecol*, 175: 970, 1996.
[120] W. Chaim, M. Mazor, T. Meril, R. Peleg, E. Maor, "Late miscarriage and intraamniotic candidiasis in a woman with a retained intrauterine contraceptive device", *Arch Gynecol Obstet*, 253: 157, 1993.
[121] D.J. Dudley, S.S. Edwin, J. Van Wagoner, N.H. Augustine, H.R. Hill, M.D. Mitchell, "Regulation of decidual cell chemokine production by group B streptococci and purified bacterial cell wall components", *Am J Obstet Gynecol*, 177: 666, 1997.
[122] K. Nakao, Y. Inoue, K. Okabe, T. Kawarabayashi, K. Kitamura, "Oxytocin enhances action potentials in pregnant human myometrium - A study with microelectrodes", *Am J Obstet Gynecol*, 177: 222, 1997.
[123] C.A. Dinarello, "Interleukin-1 and the pathogenesis of the acute - phase response", *N Engl J Med*, 311: 1413, 1984.
[124] R.G. Petersdorf, R.K. Root, "Disturbances of heat regulation", *Harrison's Principles of Internal Medicine*, McGraw - Hill Book Company, 1988.
[125] D.L. Dunn, "Use of novel therapeutic agents for the treatment of serious infection", *Am J Surg*, 166: 449, 1993.
[126] S.L. Opsjon, N.C. Wathen, S. Tingulstad, G. Wiedswang, A. Sundan, A. Waage, R. Austgulen, "Tumor necrosis factor, interleukin - 1 and interleukin - 6 in normal human pregnancy", *Am J Obstet Gynecol*, 169: 397, 1993.
[127] J.D. Palombo, P.A. Burke, L.L. Moldawer, R.A. Forse, W.D. Lewis, R.L. Jenkins, "Assessment of the cytokine response in liver donors at the time of organ procurement and association with allograft function after orthotopic transplantation", *J Am Coll Surg*, 179: 209, 1994.
[128] M. Rogy, S. Coyle, H.S. Oldenburg, C. Rock, P. Barie, K. Van Zee, C. Smith, L. Moldawer, S. Lowry, "Persistently elevated soluble tumor necrosis factor receptor and interleukin receptor antagonist levels in criticallyill patients", *J Am Coll Surg*, 178: 132, 1994.
[129] R.M. Silver, S.S. Edwin, F. Umar, D.J. Dudley, W.D. Branch, M.D. Mitchell, "Bacterial lipopolysaccharide - mediated murine fetal death: The role of interleukin – 1", *Am J Obstet Gynecol*, 176: 544, 1997a.
[130] Y. Fong, L.L. Moldawer, G.T. Shires, S.F. Lowry, "The biologic characteristics of cytokines and their implication in surgical injury", *Surg Gynecol Obstet*, 170: 363, 1990.
[131] H. Ueo, H. Inoue, M. Honda, I. Uchida, M. Nishimura, S. Arinaga, H. Nakashima, T. Akiyoshi, "Production of interleukin-6 at operative wound sites in surgical patients", *J Am Coll Surg*, 179: 327, 1994.
[132] R. Lorenz, "Clinical significance of C - reactive protein", *Scientific Department Diagnostica*, 1990.

[133] J.H. Rand, X.X. Wu, S. Guller, J. Scher, H.A.M. Andree, C.J. Lockwood, "Antiphospholipid immunoglobulin G antibodies reduce annexin - V levels on syncytiotrophoblast apical membranes and in culture media of placental villi", *Am J Obstet Gynecol*, 177: 918, 1997.

[134] W.F. Rayburn, "Prostaglandin $E_2$ gel for cervical ripening and induction of labor: a critical analysis", *Am J Obstet Gynecol*, 160: 529, 1989.

[135] A. Drazancic, S. Skrablin, V. Latin, I. Fudurik, I. Kuvacic, V. Tadic, A. Corusic, "Pregnancy outcome after early amniocentesis", *Yugosl Ginekol Perinatol*, 31: 55, 1991.

[136] N.J. Saunders, R.J. Snijders, K.H. Nicolaides, "Therapeutic amniocentesis in twin - twin transfusion syndrom apearing in the second trimester of pregnancy", *Am J Obstet Gynecol*, 166: 820, 1992.

[137] L. Borque de Larrea, A. Rus, A. Carlstrom, V. Pira, E. Delosses, P. Thiry, L. Ferrari, D. Barrozzi, G. Hafner, J. Lotz, R.J.M. van Oers, B. Leerkes, A. Szymanowicz, H. Dubois, A. Hallstein, I. Domke, "Bestimmung des C-reaktiven proteins auf Boehringer Mannheim / Hitachi Analysensystemen mit einem Verbesserten, turbidimetrischen test", *Klin Lab*, 39: 55, 1993.

[138] R.J. Wapner, A. Johnson, G. Davis, A. Urban, P. Morgan, L. Jackson, "Prenatal diagnosis in twin gestations: a comparison between second trimester amniocentesis and first trimester chorionic villus sampling", *Obstet Gynecol*, 82: 49, 1993.

[139] B.F. Crandall, P. Kulch, K. Tabsh, "Risk assessment of amniocentesis between 11 and 15 weeks: comparison to later amniocentesis controls", *Prenat Diagn*, 14: 913, 1994.

[140] Y. Romem, R. Artal, "C - reactive protein in pregnancy and in the postpartum period", *Am J Obstet Gynecol*, 151(3): 380,1985.

[141] R. Artal, "Premature rupture of membranes, in Management of common problems in obstetrics and gynecology", *Mishell DR. & Brenner PF. eds*, Blackwell Scientific Publications, Boston, 1994.

[142] St Mary's NHS Trust, *Recurrent miscarriage clinic*, 1998.

[143] C. Bulucea, N. Mastorakis, M. Paun, A. Neatu, "Medically induced abortion in second trimester with intravaginal misoprostol", North Atlantic University Union Proceedings of the World Medical Conference, 215-221, Malta, 2010

[144] R. Rai, L. Regan, E. Hadley, M. Dave, H. Cohen, "Second trimester pregnancy loss is associated with activated C resistance", *Br J Haematol*, 92: 489, 1996a.

[145] R. Rai, L. Regan, A. Chitolie, J.G. Donald, H. Cohen, "Placental thrombosis and second trimester miscarriage in association with activated protein C resistance", *Br J Obstet Gynaecol*, 103: 842, 1996c.

[146] R. Rai, L. Regan, H. Cohen, "Thrombophilic defects and pregnancy loss", *Infertility and Reproductive medicine clinics of North America*, 7(4): 745, 1996d.

[147] D.S. Dizon-Townson, L. Meline, L.M. Nelson, M. Varner, K. Ward, "Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction", *Am J Obstet Gynecol*, 177: 402, 1997.

[148] M. Kalan, D. Talbot, A.J. Rich, W.J. Cunliffe, "Evaluation of the modified Alvarado Score in the diagnosis of acute appendicitis: a prospective study", *Ann R Coll Surg Engl*, 74: 418, 1994.

[149] G.E. Welch, S.G. Gabbe, "Review of statistics usage in the American Journal of Obstetrics and Gynecology", *Am J Obstet Gynecol*, 175: 1138, 1996.

[150] C. Bulucea, N. Mastorakis, M. Paun, R. Marcu, "Circulating Lymphocyte Immunophenotypation by Flow Cytometry as Fast and Efficient Method for Immune Status Assessment in Second Trimester of Normal Pregnancy and Pregnancy Complicated by Miscarriage", *Advances in Biomedical Research*, Proceedings of the International Conference on Biochemistry and Medical Chemistry (BIOMEDCH '10), 354:524, Cambridge, 2010

[151] C. Bulucea, N. Mastorakis, M. Paun, A. Neatu, "Immunological Characterization of Late Miscarriage", *WSEAS TRANSACTIONS on Biology and Biomedicine*, Issue 3, Volume 7, 136-145, July 2010

[152] G.R. Markenson, R.K. Martin, M. Tillotson-Criss, K.S. Foley, R.S. Stewart, M. Yancey, "The use of polymerase chain reaction to detect bacteria in amniotic fluid in pregnancies complicated by preterm labor", *Am J Obstet Gynecol*, 177: 1471, 1997.