

**UNIVERSITY OF MEDICINE AND PHARMACY
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PhD THESIS

**ASSESSMENT OF PREGNANT WOMEN
WITH RISK OF PRETERM DELIVERY BY
CORRELATING SPECIFIC BIOMARKERS**

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STAGE OF KNOWLEDGE

Chapter I.

DEFINITION OF PRETERM DELIVERY AND ITS IMPACT ON THE NEWBORN CHILD

Preterm delivery represents one of the main causes of morbidity and perinatal death [186]. Further on, the prematurity is a main neonatal death cause, as well as of short term and long term morbidity cause. More than 1 out of 10 children in the world, born in 2010, were delivered before term and approximately 15 million preterm deliveries (defined as birth prior to 37 weeks of pregnancy), out of which more than 1 million children died as a result of their prematurity [36]. Prematurity is nowadays the second cause of death at infants under 5 years old and the most important death cause in the first month of life [137].

Due to the major consequences generated, it is highly necessary to find as soon as possible an adequate diagnosis and therapeutic behaviour and very well adapted for each specific patient.

The frequency of occurrence of preterm delivery is of 8-10% and may reach 25 % of the total of births in the emergent countries. In Romania the incidence of preterm delivery is of approximately 10-12% of the total of births, but in countries such as Sweden or Netherlands this pathology has an incidence of 2-5%.

Chapter II.

THE RISK FACTORS INVOLVED IN THE OCCURRENCE OF PRETERM DELIVERY

Although, in the past, up to 50% of the cases of preterm delivery were considered to be idiopathic, this may be an overestimate and with the improvements occurred in the capacity to identify the probable cause, some authors suggested to abandon up the respective notion. Despite that, in the clinical practice is most of the times impossible to determine a probable cause and, even in recent cases, the idiopathic preterm delivery may represent up to 30% of the cases [93].

The risk factors involved in the etiology of preterm delivery contain: social-economic or environmental factors; maternal factors–general; maternal factors – local; ovulatory factors.

Chapter III

ASSESSMENT OF METHODS OF IDENTIFICATION OF PREGNANT WOMEN WITH PRETERM DELIVERY RISK

The assessment of an imminence of preterm delivery is performed with the help of an ultrasound exam and of specific biochemical tests [166].

In which regards ultrasound exam, the most important method is the transvaginal ultrasound exam. In the transvaginal ultrasound between 20 and 24 weeks, it was noticed that the best cut-off was the

length of the cervix <25 mm for the occurrence of preterm delivery before 35 weeks. As an independent factor, funneling does not add an important risk to the risk of pregnancy age at the preterm delivery associated to a length of shortened cervix. So, the women with a long cervix and funneling does not represent an increased risk of preterm delivery [15].

In relation to the biochemical markers, the women with no symptoms with a positive test for fetal fibronectine have an increased risk of preterm delivery of 25% prior to 35 weeks of pregnancy, especially in the first 2 weeks from the positive result. IGFBP-1 is a predictive factor which may be used alone or in combination with other factors in order to determine the risk of a preterm delivery. The value of this globulin may orient the doctor in order to adopt a certain therapeutic behavior [174].

Chapter IV

STRATEGIES FOR THE PREVENTION OF PRETERM DELIVERY

The preterm delivery is one of the biggest challenges of obstetrics from the oldest times until the present. Due to multiple unfortunate consequences caused, the elimination of risk factors, prophylaxis and treatment of this disease represents the golden objective of current obstetrics. The treatment's purpose in case of an imminence of preterm delivery is the postponement of birth for

minimum 48 hours, in all this time taking place the transportation of pregnant woman to a specialized center and the treatment with corticoids in order to prevent the neonatal respiratory distress [62].

Chapter V

BEHAVIOR ALGORITHM IN THE CASE OF PATIENTS WITH RISK OF PRETERM DELIVERY

The standard used in the behavior algorithm in the case of patients with increased risk is represented by the prompt discovery of patients with increased and the correct diagnosis of imminence or preterm delivery under way, as well as adequate therapy and adapted for each specific patient. The behavior algorithm in the case of patients with increased risk requires a multidisciplinary attitude by collaboration between the general practitioner, obstetrics specialists, neonatologist and even the psychologist.

PERSONAL CONTRIBUTIONS

PURPOSE AND OBJECTIVES OF STUDY

The purpose of this project is the assessment of biological markers: fetal fibronectine, IL-6 and Actim Partus (phosphorylated insulin like growth factor-binding protein-1) from the cervix – vaginal secretion, associated to the clinical and ultrasound ones, which should contribute to the early discovery of the preterm delivery diagnosis.

The study performed is structured around the following *major* objectives, which represent modalities of approach in which regards in prevention of preterm delivery.

- Establishment of a bio-physical investigation group and of biological markers in order to identify preterm delivery risk groups;
- Emphasis with the help of bio-physical investigations and of biological markers the risk groups in order to establish an early diagnosis;
- Analysis of the origin and modifications in charge of the biological markers

The assessment of pregnant women based on algorithms which include, on one hand, trans-abdominal and trans-vaginal ultrasound exam and, on the other hand, biological markers shall determine the

elaboration of prognosis markers which can lead to the early detection of pregnant women with high risk of preterm delivery.

To this end, we performed three large studies on the cases studied with the risk of preterm delivery, studied in comparison to a lot of pregnancies with delivery at term.

1. *Biostatistics analysis of study lots.* The biostatistics analysis was represented by the processing of experimental data by descriptive statistics, graphic representations, tests of statistics inference, correlation studies of parameters of risk of preterm delivery and pregnancy at due term.

2. *The paraclinical assessment by ultrasound methods of certain predictive parameters for premature birth.* By corroboration of expression of morphological modifications with ultrasound aspects obtained by trans-vaginal ultrasound exam 2D and 3 D, with the maternal clinical status and risk factors could be established an investigation algorithm for the cases in which the risk of preterm delivery was present.

3. *The study of biomarkers from the level of cervix-vaginal secretion.*

The fetal fibronectine, IL-6, ActimPartus (insulin-like growth factor-binding protein-1:IGFBP-1) represented the three biomarkers studied. We intended that these potentially predictive tests for the preterm delivery to be non-invasive tests, goal achieved by the performance of ultrasound which is a non-invasive maneuver and also for the harvesting of cervix-vaginal secretion of biomarkers studied.

We analyzed in detail the comparative accuracy of various methods used for early diagnosis and diagnosis of preterm delivery. The ultrasound is largely used in Romania with obvious technical progress incorporated in the ultrasound systems frequently associated with biological markers in order to support the early diagnosis of preterm delivery.

MATERIAL AND METHOD

The prospective study performed contained a lot of 168 pregnant women with preterm delivery risk, studied in the period October 2010 – December 2012. The study was performed in the Obstetrics and Gynecology Clinic of Craiova Filantropia Municipal Clinical Hospital. During this period mentioned, the patients were introduced in the study by signing an informed consent, being subject to an investigation protocol establishing the set of quantifiable parameters, specific, for the monitoring of cases. At the beginning of the study I appreciated that there are no predictable risks for patients, and the benefits were obvious.

The study was approved by the Ethics Commission of the University of Medicine and Pharmacy of Craiova.

The pregnant women were selected pursuant to inclusion and exclusion criteria specific to the preterm delivery risk.

Definition and study procedure

I chose the performance of a screening at pregnant women with no symptoms starting with the 22-nd week of pregnancy, which represented risk factors for the preterm delivery (preterm deliveries in the personal history, multiple abortions, abortions in the 2-nd pregnancy trimester, surgical interventions at the level of the cervix - conisation, ERAD). For these pregnant women which represented risk factors for preterm delivery, the following selection criteria for screening was the *length of cervix*. I chose as cut-off a length of the cervix of 25 mm. The pregnant women which presented this parameter were, then, reassessed in an interval of 2 weeks. At the same time with the establishment of the study lot, I also began the performance of tests with prediction potential, which were represented by the Actim Partus, fetal fibronectine and IL-6 from the cervix-vaginal fluid, which were repeated within two weeks, until 27 weeks, at the same time with the measurement of cervix.

RESULTS AND DISCUSSIONS

Biostatistics analysis of the study lots

I had the following sub-lots of study in relation to the length of cervix (LC):

1. a lot containing 115 pregnant women who delivered before due term, who presented $LC < 25$ mm
2. a lot containing 32 pregnant women for which the birth took place in due time, who presented an $LC < 25$ mm
3. a lot containing 21 pregnant women who delivered before due term, but who did not present modifications of length of cervix ($LC > 25$ mm), but who had present other modified parameters taken for study.

Taking into account the age over 35 years old as maternal advanced age, we notice that the percentage of preterm deliveries was elevated, 41,51%, almost half of the pregnant women taken for study having this age delivering before due term. This observation is done in comparison to the age group 30-34 years old, at which the preterm delivery percentage was of only 10,99% and the age group 25-29 years old, with a percentage of preterm deliveries of only 7,99%. The statistics correlation of age groups showed that this parameter is significant from a statistical point of view, $p=0.017434$, so $p < 0,05$.

This observation shows that our study is correlated to the data from literature, which shows that the risk of premature delivery is more elevated at extreme ages.

We studied if between the premature births and those at due term from the study lot there is a statistical correlation in which regards the presence or absence of premature deliveries in the personal history.

By performing the p Fisher test in an accurate manner in order to compare the three lots in which the 168 pregnant women were divided, 115 cases of preterm delivery, 21 cases of preterm delivery but with $LC > 25\text{mm}$, 32 cases of delivery in due term, but with $LC < 25\text{mm}$, we noticed that the accurate p Fisher = 0.004489, that is $p < 0,05$. This shows that there is highly significant statistics correlation in which regards the presence of preterm delivery in the personal history as risk factor for the current preterm delivery.

There are speculations regarding the effects of interruption of pregnancy as an independent risk factor for the subsequent obstetrics complications. That is the reason I monitored the effect of interruption of pregnancy for the lots studied and the implication of this parameter in the occurrence of preterm delivery.

I did not find a statistically signification correlation ($p > 0,05$) between the abortions occurred in the first pregnancy trimester in the personal history and the risk of preterm delivery. In which concerns the abortions occurred in the second pregnancy trimester, these

represent a highly significant correlation from a statistical point of view ($p < 0,05$) with the preterm delivery, as presented in the specialty literature.

Another parameter belonging to the category of risk factors in this study was the determination of influence on pregnancy on the pre-pregnancy body mass index, and especially on preterm delivery. By applying the p Chi square test, $p = 0.001447$, that is $p < 0,05$, in our study MBI has a highly significant statistics importance in preterm delivery. The studies indicate that obesity may represent a risk factor for preterm delivery, but other studies did not find these types of associations.

The paraclinical assessment by ultrasound methods of potentially predictive parameters for preterm delivery

In the study performed the women for which a length of cervix ≤ 25 mm was confirmed benefited of a re-assessment of preterm delivery risk factors, as well as of the pregnancy options, although the utility of universal screening of the length of cervix in order to prevent preterm delivery is still under controversy and in debate.

Morphology and biometrics of the cervix in the prediction of preterm delivery

The length of cervix may be predictive for the risk of preterm delivery. This study's objective was to assess the length of cervix during pregnancy by transvaginal ultrasound in order to generate

normative data for pregnant women without symptoms with increased risk of preterm delivery.

In case the cervix channel is closed, LC was the only parameter measured. When the internal foramen which seemed to have a normal aspect could not be visualized, the cervix was assessed in order to establish if the funneling (internal width of the internal foramen larger than 5 mm) is present. Sludge represents an independent risk factor for the imminence of preterm delivery, chorioamnionitis and microbe invasion from the amniotic cavity for patients with spontaneous preterm delivery and intact membranes.

Several predictive signs of preterm delivery may be detected by pre-natal ultrasound, such as the presence of the area of endocervical glands. The absence of the area of endocervical glands was considered to be a predictive factor of preterm delivery for women with low risk. The absence of the area of endocervical glands reflects the advanced maturation of the cervix during pregnancy. The study's objective was to explore if an absent area of endocervical glands is a predictive factor for a preterm delivery (<34 weeks and to compare the predictive efficiency with an LC<25 mm) and the immunological tests from the cervix-vaginal secretion which were accepted.

The monitoring of pregnancies was performed by transvaginal ultrasound, starting from 22 weeks of pregnancy, within an interval of 2 weeks, in order to monitor the assessment under treatment of each pregnancy taken for study.

Length of cervix

The average pregnancy age at the admission to study was of 22,26±2,330 weeks (average ± standard deviation).

A cutoff of 25 mm (2,5 cm) was used for a primary result, because the cervix with a length of up to 25 mm is associated to the increase of risk of preterm delivery, and the pregnant women with a cervix length < 25 mm may benefit of interventions, such as cerclage, if they have certain risk factors.

The uterine cerclage was practised with emergency, in 12 cases, representing only 7,14% of the study lots, thus distributed:

- in 8 cases was performed a cerclage of the cervix with emergency, these cases having a pregnancy age at birth ranging between 30 and 36 weeks, so a preterm delivery.
- in 3 cases was performed the cerclage of cervix with emergency, these cases having a pregnancy age at birth ranging between 38 and 39 weeks, that is a delivery in term.

By practicing the uterine cerclage in cases of preterm delivery was thus obtained the extension of pregnancy age, so that we could have a sufficient fetal maturity, thus ensuring fetal viability.

The cerclage was performed at a length of cervix of 15.7 mm ± 6.188 (average ± standard deviation). The cervix was almost

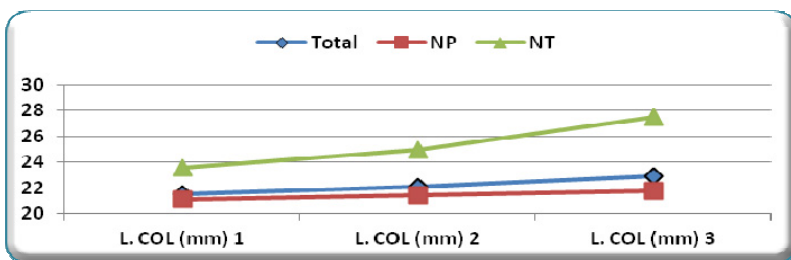
completely erased in 2 cases and a protrusion of membranes was noticed in one case.

In all cases with cerclage of cervix, we found a remarkable improvement of the ultrasound aspect of the cervix, within only a few days post-surgery, with the significant elongation of the cervix, and the reduction of funneling.

The average length of the cervix at the admission into the study was of 21,07 mm \pm 3,32 standard deviation, for the pregnant women who delivered before due term and of 23,53% \pm 1,14 standard deviation, for the pregnant women who had a cervix of <25 mm, but who delivered in due term.

For the lot studied, we encountered a length of the cervix of < 25 mm at 84,56% of the cases in which the women delivered before due term and in only 2,58% for the cases in which they delivered in due time.

We identified a highly significant difference of distribution between the preterm deliveries and the deliveries in due term, in relation to the length of the cervix being larger or smaller than the threshold value of 25 de mm. The p value corresponding to the Chi square test was smaller than 0,001, so we can state that we have a significantly larger percentage of patients with LC <25 mm in the group of those who had preterm deliveries rather than in the group of those with deliveries within due term.



The schematic representation of the elongation of cervix in pregnant women who delivered before due term and in due term

Funneling

In percentage, the ultrasound aspect of funneling was more elevated in pregnant women who delivered preterm, 28,68%, in comparison to the pregnant women who delivered in due term, but had a LC < 25 mm, 2,74%.

We identified a highly significant difference of distribution between the preterm deliveries and those in due term, in relation to the occurrence of the funneling phenomenon, the accurate result of Chi square test being of $p \sim 0$, that is under the threshold of 0,001. In conclusion, for the population from which the lot of studied patient originated, we have a significantly higher percentage of funneling in patients who had preterm deliveries rather than at those which deliveries in due term.

As an independent factor, the funneling aspect does not significantly increase the risk of preterm delivery, with the exception of association with a shortened cervix length. So the women with a long cervix and funneling do not present a high risk of preterm delivery.

The sludge of amniotic liquid was also reported as being an independent risk factor for preterm delivery. In association with a shortened cervix, the studies correlated this aspect with a higher risk for preterm delivery rather than the risk noticed with the independent variable. In the current study, we encountered only 13 cases (9,56%) presenting sludge in the lot of pregnant women who delivered before due term and only 1 (1,69%) case associated with a delivery within due time, but with long cervix.

The area of endocervical glands

The ultrasound absence of the area of endocervical glands reflects the maturation of cervix and might be considered as a predictive aspect of the threat of preterm labor.

From among the pregnant women who delivered before due time, at < 34 weeks, 27 cases (19,85%) presented absence of the area of endocervical glands, and the pregnant women who delivered in due term, only 13 cases (7,73% of the total lots of study) had the area of endocervical glands absent.

In order to determine the efficiency of screening for the ultrasound method, as an independent factor of predictability, we monitored a series of specific statistics parameters.

Although each test has its own performance measurement and adequate use, the clinical tests are designed in order to be equally accurate, exact, specific and sensitive, as much as possible. These basic concepts are the headstones of reliability of tests, thus offering trust to the suppliers of health in their clinical use.

Efficiency of ultrasound screening for the prediction of preterm delivery

Parameter	Ac (IC 95%)	Sn (IC 95%)	Sp (IC 95%)	VPP (IC 95%)	VPN (IC 95%)	LR+ (IC 95%)	LR- (IC 95%)
LC	96.15%	84.56%	97.42%	78.23%	98.29%	32.81939	0.004705
Funneling	38.10%	28.68%	97.26%	53.42%	92.57%	10.47534602	0.068087039
Sludge	89.55%	9.56%	98.31%	38.24%	90.85%	5.653361345	0.159977703
Area of endocervical glands	89.62%	29.41%	96.22%	45.98%	92.56%	7.772215269	0.090821256

Ac: accuracy; Sn: sensitivity; Sp: specificity; VPP: predictive positive value; VPN: predictive negative value; LR+: positive likelihood; LR-: negative likelihood ratio; IC: confidence interval

In order to verify if the use of cervix's length under 25 mm represents a pertinent diagnosis test for the identification of preterm deliveries, we calculated the sensitivity and specificity of such test on the lot studied. We obtained a higher sensitivity, of 84,56%, as well as a very high specificity, of 97,42%, the precision of this test being of 96,15%.

As a consequence, the use of classification of patients in relation to the length of the cervix, taking as threshold the value of 25 mm, represents a useful marker for the diagnosis of preterm deliveries. Theoretically, there are 32,82 more chances (LR+) to encounter a preterm delivery when we identify a cervix shorter than 25 mm than when the length is larger than 25 mm.

As practical utility, the predictive positive value of 78,23% shows that, in the case of identification of a cervix with the length <25mm in the second trimester of pregnancy, there is a probability of over 75% to have a preterm delivery, meanwhile the predictive negative value shows that, if we do not identify a cervix < 25mm, the

probability to deliver in due time is of 98,29% – in other words, the probability of preterm delivery is of only 1,71%

One of the objectives analyzed was the presence of funneling at the level of cervix. The accuracy of this statistics comparison was of 90,49%, which shows that the presence of funnelling is a highly accurate parameter for the prediction of preterm birth. The specificity of this ultrasound parameter is high, 97,26%, but the sensitivity is lower, but sufficiently high, of 28,68%, in order to affirm that this ultrasound parameter has a significant value in assessing the risk of preterm delivery. These considerations are also supported by the predictive positive value, which exceeds 50%, that is 53,42%, this being the percentage which shall characterize the pregnant women with risk who shall deliver before their term. The negative predictive value of 92,57% shows that, in the conditions of absence of funneling, the possibility that a pregnant woman with risk to deliver in due time is very high.

As practical use, the predictive positive value of 53,42% shows the fact that, in case of identification of the phenomenon of funneling, there is a probability of over 50% to have a preterm delivery, meanwhile the predictive negative value shows that, if we do not identify the funneling, the probability to deliver in due term is of over 90% (92,57%) – in other words, the probability of preterm delivery is of only 8,43%.

Another objective analyzed was the presence of sludge at the level of amniotic cavity. Although the specificity of this test is of 98,31%, the sensitivity is pretty small, of 9.56%, which indicates that

this is not a diagnosis test with high significant value in assessing the preterm delivery risk. This is emphasized also by the predictive positive value of under 50% (38,24%). The negative predictive value is over 90%, which leads us to emphasize that in the conditions of the absence of sludge at the ultrasound exam, that is of an amniotic infection, the probability that the patient shall deliver on due term is of 90.85%.

The positive probability report warns us that the pregnant women who have sludge present at the ultrasound exam, have a preterm delivery risk, of more than 5 times larger in comparison to the rest of the pregnant women who do not present negative probability report shows us that they have a risk of only 0,15% for preterm delivery.

The first ultrasound parameter investigated was the presence/absence of endocervical glands area.

The accuracy of the statistics comparison was of 89,62%, which leads to the conclusion that it is a parameter with a high accuracy. The specificity of this ultrasound parameter is high, 96,22%, and the sensitivity is high, of 29,41%, in order to assess if this parameter has a sufficiently significant value in assessing the risk of preterm delivery. But the predictive positive value of under 50% (45,98%), shows us that this parameter cannot be used as an independent predictive risk factor. The negative predictive value is of over 90%, which makes us emphasize that, in the conditions of the presence of the area of endocervical glands at the ultrasound exam, the probability that the patient shall deliver within due time is of 92,56%.

The positive probability report warns us that the pregnant women who do not have present the area of endocervical glands at the ultrasound exam have a risk of preterm delivery of over 7 times higher in comparison to the rest of the pregnant women presenting this ultrasound parameter and that which the negative probability report shows us that they have a risk of only 0,09% for preterm delivery.

Study of biomarkers from the level of cervical-vaginal secretion

The cervical-vaginal fluid has an important function in the homeostasis and immunity of inferior feminine genital tract. The analysis of cervical-vaginal liquid may, as such, bring important information regarding the pathogenicity of numerous gynecology pathologies. In addition, the cervical-vaginal liquid has a high potential as source of biomarkers for these conditions.

Fetal fibronectin

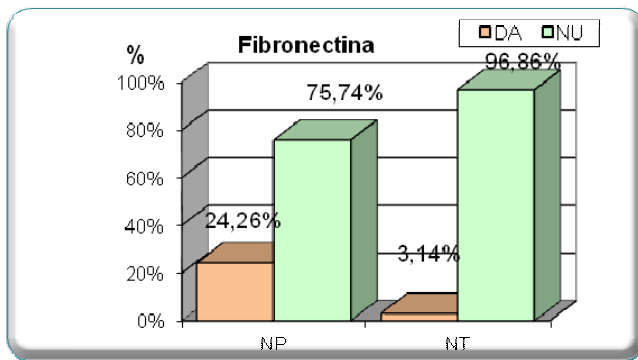
From a physiological point of view, FF is found in the cervical-vaginal secretions in the first 22 months of pregnancy, but disappears after the 24th and 34th weeks of pregnancy, except the cases with eroded or preterm dilatation cervix, associated with symptomatic uterine contractions.

A positive value of FF was encountered at 24,26% of the pregnant women without symptoms, with risk of preterm delivery, who also subsequently delivered preterm. Only 3,14% of the

pregnant women who had an evolution of pregnancy until the term presented a positive value of FF.

Our lot presented only 11 pregnant women (6,54%) who prematurely gave birth at a pregnancy age of < 34 weeks + 0 days. The explanation might be that the patients without symptoms may develop the subsequently identifiable risk during pregnancy, even if the result of the FF test is negative at 24-26 weeks of pregnancy.

A high negative predictive value of FF in the cervical-vaginal secretions may be clinically used in order to reduce to minimum the potentially dangerous or expensive surgeries, such as the tertiary transfer, hospitalization, administration of corticosteroids and tocolytics.

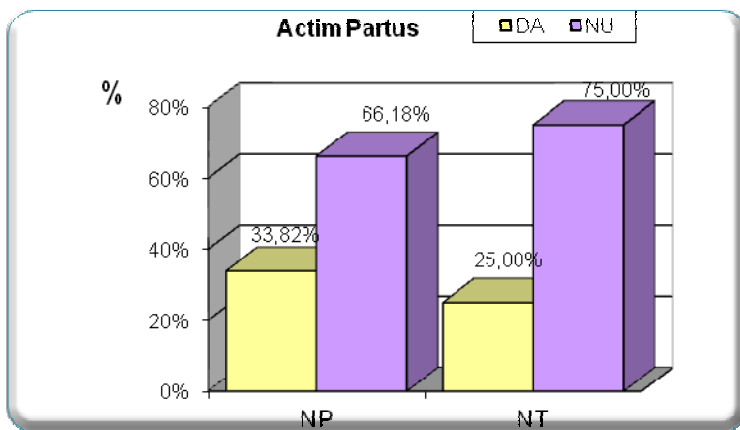


Percentage distribution of the fetal fibronectine test at the lost studied

ActimPartus test (IGFBP-1)

The test's calibration is performed to a cut-off of 10 microg/L. We considered that, if the concentration of IGFBP-1 is <10 microg/L (ActimPartus negative test) at pregnant women with no symptoms, the risk of preterm delivery is decreased.

In our study we encountered at the lot of pregnant women who delivered preterm a number of 46 pregnant women (33,82%) who presented a positive Actim Partus test. At the lot of pregnant women who delivered preterm after 37 weeks, we encountered only 8 such cases, representing a percentage of 25,00%.



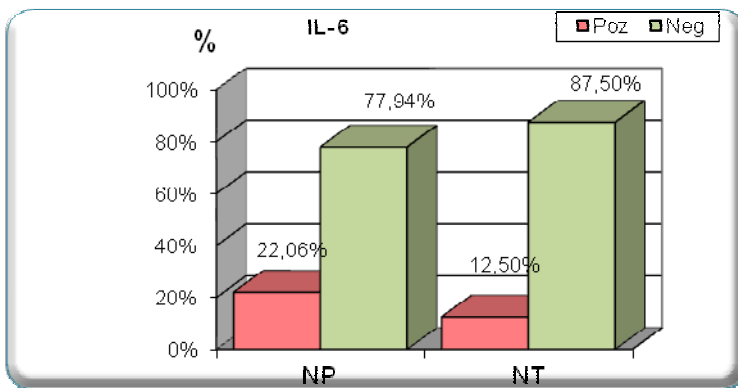
Percentage distribution of ActimPartus test for the lots studied

Our test shows that, if at the level of cervix pHIGFBP-1 is identified through the immuno-chromatograph formula, it is a rapid and easy to apply test, which may anticipate the imminence of preterm delivery at patients with risk.

Thus, the vaginal identification of pHIGFBP-1 seems to be a favourable option in identifying the pregnant women with no symptoms, with risk of preterm delivery and in the management of patients with no symptoms, taking into account an important advantage of IGFBP1-P – its low cost.

Dosage of IL-6 in cervical-vaginal secretion

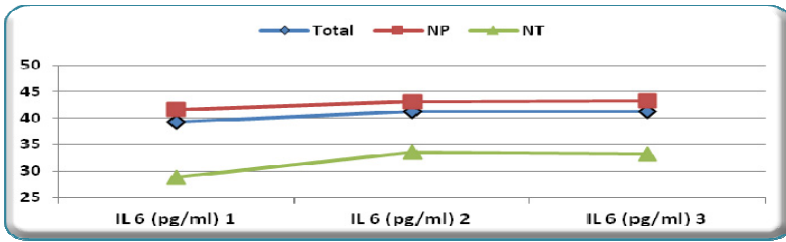
The use of cervical-vaginal secretion was suggested as an alternative to the testing of amniotic liquid, because this liquid is released through mechanic deterioration or mediate inflammation in membranes or placenta prior to birth. I considered that a value of IL-6 <50pg/ml may be considered negative, pursuant to the standard curve of the kit of IL-6 used, as well as of the specialty data.



The percentage distribution of cervical-vaginal dosage of IL-6 at the lots studied

We noticed that the average of cervical-vaginal values of IL-6 was higher at the lot of pregnant women who delivered preterm rather than at the lot of pregnant women who delivered in due term.

We may notice that, in comparison to the second assessment, the average values, as well as the maximum and minimum values, did not modify very much, which makes us conclude that a harvesting of cervical-vaginal IL-6 at a pregnancy age of approximately 26-28 weeks, would be beneficial for the predictive aspect of this test.



The evolution of cervical-vaginal values IL-6 at the three harvestings

The efficiency of screening of biomarkers for the prediction of preterm delivery

In order to determine the efficiency of the screening through the assessment of specific biomarkers from the level of endocervical secretion, as independent predictability factors, we monitored a series of specific statistics parameters.

The non-invasive tests to which we refer were as follows: the test of fetal fibronectine, the ActimPartus test and the IL-6 dosage, all these being harvested from the level of cervix, from the cervical-vaginal secretion.

Efficiency of screening through specific biomarkers for the prediction of preterm delivery

Parameter	Ac (IC 95%)	Sn (IC 95%)	Sp (IC 95%)	VPP (IC 95%)	VPN (IC 95%)	LR+ (IC 95%)	LR- (IC 95%)
Fetal fibronectine	89.70%	24.26%	96.86%	45.83%	92.11%	7.727376	0.098009
Actim Partus	89.26%	33.82%	95.33%	44.23%	92.94%	7.242901	0.091367
IL-6	91.36%	22.06%	98.95%	69.77%	92.06%	21.07466	0.036983

Ac: accuracy; Sn: sensitivity; Sp: specificity; VPP: positive predictable value; VPN: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; IC: confidence interval

One of the tests analyzed was the presence/absence of fetal fibronectine through its dosage at the level of cervix. The accuracy of this statistics comparison was of 89,70%, which shows that the presence of fetal fibronectine is a parameter with high accuracy for the prediction of preterm delivery. The specificity of the test of fetal fibronectine is very high, 98,86%, but the sensitivity is lower, 24,26%, but sufficiently high in order to assess that this parameter has a value in the evaluation of the preterm delivery risk.

The negative predictive value of 92,11% shows that, in the conditions the test of fetal fibronectine is negative, the probability for a pregnant woman with risk to deliver in due term is very high.

The likelihood ratio (LR+) show sus that the pregnant women who have a positive fetal fibronectine test have a risk of preterm delivery, of almost 8 times larger, compared to the pregnant women presenting a negative fetal fibronectine test and at which the negative likelihood ratio (LR-) shows us that they have a risk of only 0,09% of preterm delivery.

Another parameter analyzed was the identification at the level of cervix of pHIGFBP-1, Actim Partus test. The accuracy of this test was of 89,26%, which leads to the conclusion that the ActimPartus test is a parameter with a very high accuracy. This test's specificity is of 95,33%, the sensitivity is also sufficiently high, of 33,82%, which indicates the fact that this test is a predictive test of high significance

in the assessment of the preterm delivery risk. This fact is also emphasized by the increased positive likelihood, of 44,23%.

The third parameter used was only the dosage IL-6 from the level of the cervix, from the endocervical secretion. The specificity of this parameter is high, 98,95%, and the sensitivity even not as high as at the other two tests, is sufficiently elevated, in value of 22,06% in order to assess if this ultrasound parameter has a sufficiently significant value in assessing the risk of preterm delivery. The predictive positive value of over 50% (69,77%) shows us that this parameter may be used as an independent risk prediction factor.

The predictive negative value is over 90%, which allows us to emphasize that, in the conditions of a value below 50 pg/ml, which represents a negative test, the probability that the patient shall deliver in due term being of 92,06%.

Due to the fact that we demonstrated that the identification of a length of cervix of below 25 mm represents a good test for the prediction of preterm deliveries, we wish to analyze if the use of an additional factor may improve the estimate obtained in this manner.

This is the reason why we assessed at the sub-lot of patients with the length of cervix under 25 mm the influence of the other ultrasound parameters or biological markers in determining the preterm deliveries.

The statistics correlation of ultrasound parameters with biomarkers at the lot studie dat the first assessment

NP/ NT	Sensitivity	VPP	p Fisher	Odds Ratio	Likelihood interval for OR	
Funneling	29.57%	82.93%	0.267	1.499	0.592	3.795
Sludge	11.30%	92.86%	0.144	3.951	0.497	31.415
ActimPartus	30.43%	81.40%	0.359	1.313	0.537	3.207
Fibronectin	28.70%	89.19%	0.046	2.817	0.917	8.659
LC	21.74%	86.21%	0.183	1.944	0.623	6.065

The only connection more important, but a bit less under the maximum threshold which still shows statistics significance, was identified for fibronectine (p test Fisher = 0,046), but, neither in this case the value of Odss Ratio was not significant from a statistics point of view (OR =2,817), but the likelihood interval 95% = 0.917 – 8.659 contains value 1, which shows the absence of an influence between the two factors, the preterm delivery and the positive fibronectine.

Thus, at the first assessment, the dosage of fibronectine does not give us certain data in which regards in late preterm delivery, the results being predictive only for 7, 14 days, in this period speaking about the abortion.

The statistics correlation of ultrasound parameters with biomarkers at the lot studied at the third assessment

NP/ NT	Sensitivity	VPP	p Fisher	Odds Ratio	Likelihood interval for OR	
Funneling	19.13%	88.00%	0.150	2.287	0.638	8.194
Sludge	7.83%	90.00%	0.315	2.632	0.321	21.589
ActimPartus	23.48%	79.41%	0.528	1.096	0.427	2.812
Fibronectine	27.83%	96.97%	0.001	11.952	1.565	91.248
LC	21.74%	86.21%	0.183	1.944	0.623	6.065

The third assessment does not present important modifications, the indexes for fibronectine being the only ones who show a stronger connection to the preterm delivery, this time almost highly significant from a statistics point of view, (p Fisher test = 0,00124). Now also Odds Ratio has a significant statistics value, OR = 11,952 (likelihood value 95% = 1.565 – 91.248).

For the sub-lot of patients who presented at the initial investigation values LC<25mm we wanted to establish if there are significant correlations from a statistics point of view between the biological markers monitored. In order to verify the statistics connection between the results obtained by testing the biological markers, we used the Fisher exact test, which shows the dependence between the two qualitative variables (ordinary or nominal type).

By performing the statistics analysis, we established that there are significant connections between the test for fetal fibronectine and the one for ActimPartus at all the 3 assessment moments, thus:

- at the initial assessment, we obtained p test Fisher = 0,000882 - <0,001, so we have a highly significant connection

- at the second assessment, we obtained p test Fisher = 0,000992 - <0,001, so we have a highly significant connection

- at the third assessment, we obtained p test Fisher = 0,001482 - <0,01, so we have a significant connection, with a likelihood of 99%

Although the connection between the results for fetal fibronectine and the ones for Actim Partus is certain, the degree of compliance between these results, close to 50%, is not one to allow giving up to one of the tests and only the performance of the other.

Compliance of ActimPartus with FF

Concordanța	% din FF+ au AP+	% din AP+ au FF+
Eval 1	44.19%	51.35%
Eval 2	45.00%	48.65%
Eval 3	44.12%	45.45%

In case IL-6, I did not obtain any significant association with Actim Partus or with fetal fibronectine.

In conclusion, we may affirm that these three biological markers are independent and that it would be necessary to perform each of them for an identification as accurate as possible of the patients with preterm delivery risk.

FINAL CONCLUSIONS

- ❖ There is a highly significant difference, that is $p < 0,05$ between the distribution on age groups of pregnant women who had preterm deliveries, respectively deliveries in due time, that is the preterm delivery pregnant women tend to be older, over 35 years old (41,51%), than those with deliveries in due term.
- ❖ The personal history of preterm delivery represents a strong risk factor for the preterm delivery at the current pregnancy, if also correlated with $LC < 25$ mm, the statistics verification showing that $p < 0.05$. This parameter as risk factor is unanimously accepted as being an important factor in causing preterm delivery.
- ❖ In all cases of cerclage of cervix in the second trimester of pregnancy, I found a remarkable improvement of the ultrasound aspect of the cervix, at just a few days following the surgery, with the significant elongation of the cervix and reduction of funneling.
- ❖ As practical use, the predictive positive value of LC of 78,23% shows that, in the case of identification of a cervix with the length of < 25 mm in the second trimester of pregnancy, there is a probability of over 75% to have a preterm delivery, meanwhile the predictive negative value

shows that, if we do not identify a cervix $<25\text{mm}$, the probability to deliver in due term is of 98,29% – in other words, the probability of preterm delivery is of only 1,71%.

- ❖ The funneling aspect is predictive for the preterm delivery, taken as an individual risk factor, but only associated to a short cervix. The predictive negative value of 92,57% shows that, in the conditions of absence of funneling, the probability for a pregnant woman with risk to deliver in due time is very high.
- ❖ The specificity of presence of the phenomenon of sludge is of 98,31%, the sensitivity is pretty low, of 9.56%, which indicates the fact that this is not a diagnosis test of a highly significant value in assessing the risk of preterm labor. This is also emphasized by the predictive positive value of under 50% (38,24%).
- ❖ I encountered the absence of the area of endocervical glands in 70,59% of the cases at the lot of pregnant women who delivered before the due term. The specificity of this ultrasound parameter is high, 96,22%, and the sensitivity is sufficiently elevated, of 29,41%, but the predictive positive value of under 50% (45,98%) show sus that this parameter cannot be used as an independent predictive risk factor.
- ❖ A predictive negative value of FF in the cervical-vaginal secretions may be clinically used in order to reduce to the

minimum the potentially dangerous or expensive interventions, such as the tertiary transfer, hospitalization, administration of corticosteroids and tocolytics. The predictive negative value of 92,11%, shows that, in the case the fetal fibronectine test is negative, the probability that a pregnant woman with risk shall deliver in due term is very high.

- ❖ The specificity of the test Actim Partus is of 95,33%, the sensitivity is also sufficiently high, of 33,82%, which indicates that this test is a significantly high value predictive test in assessing the risk of preterm delivery. This is also emphasized by the elevated predictive positive value, of 44,23%.
- ❖ The specificity of dosage IL-6 at the level of cervix is high, 98,95%, and the sensitivity, even if not as high as at the other two tests (22,06%), in order to assess that this ultrasound parameter has a sufficiently significant value in assessing the risk of preterm delivery.
- ❖ The statistics correlation between the ultrasound measurement of the cervix and the biomarkers used showed us that there is a certain link between LC and the results for fetal fibronectine and Actim Partus, the degree of compliance between these two results being close to 50%.
- ❖ In the case IL-6, I did not obtain any significant association with Actim Partus or with fetal fibronectin.

- ❖ The use of screening of the length of cervix as an identification method of a large number of women who might benefit of intervention in the case of preterm delivery risk might cause a significant impact on the general reduction of the preterm delivery ratio.
- ❖ The use of these techniques might help to the prevention of unuseful interventions for the women with reduced risk and the acceleration of application of therapy for women who present the highest risk of premature delivery.

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