B.M. Gasanova, M.L. Polina, N.I. Douglas

PREDICTON OF REPRODUCTIVE LOSSES IN THE FIRST TRIMESTER IN WOMEN WITH CHRONIC PYELONEPHRITIS AND ANEMIA

DOI 10.25789/YMJ.2021.75.15

УДК 61.618.2

After a comprehensive examination of a sample of pregnant women with anemia and chronic pyelonephritis, the risk factors for belonging to groups with a high infectious risk in the first trimester were identified in this sample. The risk of reproductive losses is determined by angiopathy of uterine vessels, supplemented by a disruption of homeostasis and violation of the microbiome of the urogenital tract in the absence of pregravid recovery and prevention of placental insufficiency.

Keywords: iron deficiency anemia, anemia of chronic diseases, chronic pyelonephritis, reproductive losses, risk factors.

The period of embryogenesis, early fetogenesis and placenta formation is actively studied as important for pregnancy outcomes and the most vulnerable to various factors.

Predicting gestational complications in women with anemia and chronic pyelonephritis (CP) is impossible in the absence of clear ideas about the features of the uterine-fetal interaction at the time of cytotrophoblast invasion into the walls of the spiral arteries. The inability to fully remodel the spiral arteries of the uterus into vessels with low vascular resistance leads to abnormal initiation of the villous trophoblast and impaired placental perfusion [8].

The issues of pathophysiological adaptation of the embryo / fetus in women with anemia and CP have been poorly studied from the standpoint of identifying groups with a high infectious risk and predicting adverse pregnancy outcomes from miscarriage to the birth of an intrauterine infected child [4,13,16,20].

An increase in obstetric and perinatal complications is associated with iron deficiency (ID) on the background of infectious and inflammatory diseases [1,9].

Understanding the mechanisms of chronic placental ischemia and its consequences in pregnant women with extragenital diseases (EGD) is connected to the possibility of early diagnosis of potential fetal problems [6,15].

The issues of the course of pregnancy in women with EGD are inherently related to the clarification of the characteristics of the urogenital microbiome, immune and metabolic reserves of the body, the presence / activity of infectious and inflammatory processes [7,27].

The influence of the timing of ID manifestation and the severity of anemia on the outcomes of pregnancy is debated [11]. The development of anemia in the third trimester is associated with a lower frequency of prematurity and low weight [22]. There is practically no data in the literature on the effect of anemia on the probability of reproductive losses (RL) in the first trimester. The expansion of ideas about the possibilities of a real influence on the formation of an early placenta and the preservation of the physiological foundations of the embryo-placental interaction is achievable by identifying predictors of critical trophic and metabolic disorders and the possibility of real overcoming the termination of pregnancy in the early stages. The effect of the timing of the start of therapeutic and preventive measures on the course of pregnancy in women with CP and anemia is also not specified.

The aim of the study was to determine risk factors and prognostic criteria for termination of pregnancy in women with a high infectious risk caused by extragenital diseases (anemia, chronic pyelonephritis).

Material and methods. Women with CP (n = 320) and anemia (n = 308) were retrospectively divided into groups depending on pregnancy outcomes: with progression and termination in the first trimester (by the type of undeveloped pregnancy or miscarriage) (with CP n = 135 and n = 185 respectively, and anemia n = 62 and n = 246, respectively).

We identified pregnant women with iron deficiency anemia (IDA) (n = 108) and anemia chronic diseases (n = 200).

Inclusion criteria: singleton progressive pregnancy; the woman's informed consent for the use of biological material for scientific purposes, extragenital diseases found before pregnancy and confirmed by specialists (CP, anemia).

Exclusion criteria: multiple pregnancies and those resulting from assisted reproductive technologies; severe somatic diseases in the decompensation stage, precancerous and oncological diseases; stillbirths; chromosomal abnormalities and congenital malformations of the fetus.

Methods of research: laboratory (general analysis of blood, urine, assessment of iron metabolism (serum, ferritin, transferrin, total iron-binding capacity (TIBC) of serum), microbiological (smear microscopy for the degree of purity (Gram stain), bacteriological inoculation of cervical discharge for flora and sensitivity to antibiotics, polymerase chain reaction (PCR study), molecular genetic research (Femoflor). Microscopy and culture examination of urine were performed as part of screening, in the presence of bacteria or dysuria in the urinary sediment, pain in the lumbar area on a set of standard media (5% blood agar, Endo medium, Sabouraud, MRS-arap), with incubation in aerobic conditions at 37°C for 18 hours.

Sonography of the fetus and placenta with dopplerometry was performed on expert class devices Voluson E8, Tochiba Aplio XG.

The obtained endometrial tissue samples were fixed with 10% formalin solution for 24 h, embedded in paraffin, sections were prepared with a thickness of 6 microns, and stained with hematoxylin and eosin.

The level of production of placental proteins (pregnancy-associated protein (PAPP-A), placental lactogen) was assessed.

Immunoreactivity was studied based

GASANOVA Bakhtykei Musalavovna – PhD in Medical Sciences, Doctoral Candidate of the Department of Obstetrics and Gynaecology of the Medical Institute of the Federal State Autonomous Educational Institution of Higher Education People's Friendship University of Russia, Moscow, Russia, https://orcid.org/0000-0001-6871-7102; **POLINA Miroslava Leonidovna** – PhD in Medical Sciences, Gynecologist at the Women's Health Medical Center, Moscow, Russia, polina.ml@mail.ru https://orcid.org/0000-0003-3883-3967; **DOUGLAS Natalya Ivanovna** – M.D., Head of the Department of Obstetrics and Gynecology, Medical Institute of North-Eastern Federal University, Yakutsk, Russia.

on the results of ELISA-detected Probably of pathology, an enzyme-linked immunosorbent assay of the number and affinity of individual embryotropic autoantibodies interacting with embryogenesis regulatory proteins (Biopharm – test LLC).

The degree of reliability and approbation of the results of the work. The statistical analysis was carried out using the IBM SPSS Statistics 23 program, parametric analysis methods in accordance with the results of checking the compared masses for the normality of distribution, descriptive statistics (arithmetic mean (M), mean error of the mean (m), Student's t-test, odds ratio (OR), confidence interval (CI, 95%).

The analysis of intergroup differences in qualitative characteristics was carried out using the criterion χ2, less than five – the exact two-sided Fischer test. The significance level (p) when testing statistical hypotheses was taken to be p≤0.05.

The construction of a predictive model for calculating the risk was performed using the binary logistic regression method according to the formula:

$$P = \frac{1}{1+e^{-z}}, z = a0 + a1x1 + \\ + a2x2 + a3x3 + \ldots + anxn,$$

where p is the probability of the outcome, x1… xn are the values of the predictors in a nominal, ordinal or quantitative scale, a1… an are the regression coefficients, using Wald statistics. The effectiveness (the proportion of correctly predicted cases of the presence and absence of the studied pathology), sensitivity (the presence of pathology), specificity (the absence of pathology), the prognostic value of a negative result (PVNR) and a positive one (PVPR) were determined, ROC analysis (receiver operating characteristic) of the error curve was done. The Area Under Curve (AUC) under the ROC curve was calculated.

Results and discussion. The effectiveness of pregravid rehabilitation of women with EGD is proved by a lower incidence of infectious and inflammatory diseases during pregnancy: anemia chronic diseases in comparison with IDA (28,6% vs 59.0%, p=0.03), exacerbation of CP one and a half times more often than cases of remission of the disease (39,4% vs 60.7%, p=0.02).

Treatment and preventive measures during the first wave of placentation were taken in every fifth pregnant woman with CP (21,9%), IDA – 4.5 times more often than with anemia chronic diseases (p=0.005).

According to the calculations of logistic regression, markers that determine the belonging of pregnant women with anemia and CP to the group with a high infectious risk were: the absence of pregravid preparation (χ2 = 8.6; p = 0.003), the total absence of the pregravid stage and the early prevention of placental insufficiency (χ2 = 12 , 3; p = 0.000) (Table 1).

The effectiveness of the predictive model for identifying patients with a high infectious risk in the sample with EGD (CP and anemia) is reflected by the data: regression coefficient B – 1.22; Wald statistic χ2 – 57.9; p – 0.00; Exp B – 3.4, Nagelkirk exponent – 0.39.

The area under the curve AUC-0.82 allows us to regard it as reliable. The value at the "cut-off" point – 0.5 – shows the positive predictive significance of the model when the value is exceeded. Model accuracy assessment is presented as the following: sensitivity – 95.9%; specificity – accurately predicted – 40.0%. The diagnostic efficiency of the logistic model, determined by the proportion of all correct predictions, is 83.2%. PVRP (the prognostic value of a positive result – 95.9%, PVNR (the prognostic value of a negative result) – 40.0%.

Such conclusions allow us to assert that proper pregnancy management in women with EGD (CP, anemia) begins not from early stages, but from pregravid recovery.

According to the logistic regression data, belonging to the group with a high infectious risk was determined by the fact of CP exacerbation, with an increase in temperature on the background of inflammatory criteria (leukocyturia, proteinuria, hematuria), mainly in the absence of timely antibacterial therapy for the detection of asymptomatic bacteriuria (AB).

CP exacerbation occurred in 7.5% of women in the first trimester (χ2=8,759; p=0.003), with bacteriuria – in all pregnant women.

The probability of developing unfavorable obstetric outcomes with the lack of rational management tactics for pregnant women with CP was confirmed by other authors. The expediency of treating urinary tract infections [26], the prevalence of which in pregnant women reaches 10%, is explained by the risk of CP exacerbation [17]. Asymptomatic bacteriuria (AB) requires treatment to avoid the risk of developing acute pyelonephritis in 25-30% [14]. The formation of the focus of inflammation in persistent infection is determined by the ability of uropathogenic strains to synthesize virulence factors and damage of kidney tissues [24].

According to the logistic regression data, moderate anemia of pregnant women is among the features that presuppose belonging to a high infectious risk group.

Moderate anemia was detected in 12 women (37.5%) from the group with a high infectious risk (n = 32), with reproductive losses in the first trimester. In the sample with prolonged pregnancy, the disease was noted in 14.5% of women without a high infectious potential.

In total, moderate anemia in the first trimester of pregnancy was identified in 39.8% of women.

The effect of moderate anemia on pregnancy outcomes is explained by a number of factors: decreased bioavailability of iron and a violation of its transfer through the placenta to the fetus [25]. Programming the rate of fetal development in ID is explained by the deterioration of vascularization on the background of a violation of the primordial trophoblast initiation and the formation of primary placental insufficiency [21]. Timely and adequate therapy of anemia, taking into account its genesis, is most significant due to the correlation of ID with chronic infectious and inflammatory diseases of the genitourinary system.

According to the calculations of the logistic model, the cumulation of miscarriage risks with EGD is determined in the presence of the features presented in Table 2.

The high prognostic significance of the model is proved by the following criteria: sensitivity – 91.8%, specificity – 46.0%, diagnostic efficiency – 81.4%, PVPR – 91.8%, PVNR – 46.0%. The area under the AUC curve – 0.94, "cut-off" – 0.5 – shows a high predictive value of the model when the indicator is exceeded.

Pregnant women with CP and anemia in the group with RL were distinguished by increased microbial contamination of the genital tract in comparison with a favorable pregnancy result: bacterial vaginosis (38.5% versus 25.9%, p = 0.002) and infection of the cervical canal with E. coli (24.4% against 13.2%, p = 0.001), Str. haemolyticus (14.2% versus 5.1%, p = 0.0006), Str. epidermalis (11.4% versus 5.6%, p = 0.03).

"Disruptions" of local infectious protection in women with abortion due to CP and anemia lead to a high level of bacterial contamination of the cervical canal: in CP – Mycoplasma genitalium (23.2% versus 12.2%, p = 0.005) and Ureaplasma urealyticum (32, 4% versus 14.2%, p = 0.005) – 2 and 2.3 times more often than in anemia. Women with reproductive losses (RL) on the anemia background were distinguished by a high frequency of Gardnerella vaginalis (27.2% versus

Table 1

Factors determining the belonging of pregnant women with CP and anemia to the group of a high infectious risk

Factors	Regression coefficient B	Wald statistic, χ^2	Value, p	Exp B
Multiple intrauterine surgeries	1.126	7.024	0.008	3.085
Absence of pregravid preparation	1.596	8.604	0.003	4.936
Absence of pregravid preparation / early prevention of placental insufficiency	1.517	12.307	0.000	4.558
Exacerbation of chronic pyelonephritis	1.496	8.759	0.003	4.465
Moderate pregnancy anemia	1.788	18.080	0.000	5.976
Constant	-1.231	10.070	0.002	0.292

Table 2

Risk factors for miscarriage in the first trimester of pregnancy on the background of a high infectious risk in women with CP and anemia

Factors	Regression coefficient B	Wald statistic, χ^2	Value, p	Exp B
Relapses of bacterial vaginosis, contamination of the cervical canal	1.5	8.4	0.004	4.7
Contamination of the cervical canal, urine	0.9	3.9	0.049	2.5
Hyporeactivity	1.4	4.9	0.03	3.9
pulsation index in the uterine arteries >1,5 at 6-8 weeks	1.7	9.1	0.003	5.3
Placental lactogen, mg / l <1.4 (min 1.5 times less than the initial)	1.1	4.3	0.04	2.9
PAPP-A>620, mU / l (min 1.3 times more than the initial)	1.9	8.4	0.004	6.8
Constant	-0.7	5.2	0.02	0.5

16.8%, p = 0.01) – almost 1.5 times more often than with CP.

Asymptomatic bacteriuria (AB) in women with abortion was detected 2.6 times more often than with progression (19.4% versus 7.4%) due to Escherichia coli (45.8%), Klebsiella pneumonia (15.3%), Proteus mirabilis and Staphylococcus spp. (9.7% each), which confirmed the need for repeated bacteriological examination of urine during pregnancy (Mancia G. et al., 2013; Tan M.Y. et al., 2018).

Thus, the probability of pregnancy termination in the first trimester increased in the presence of recurrent bacterial vaginosis (BV) and contamination of the cervical canal (p = 0.004, χ2 = 8.4), simultaneous persistence of infections in the cervical canal and urine (p = 0.0049, χ2 = 3.9).

The frequency of reduced production of embryotropic autoantibodies in groups with reproductive losses (RL) on the EGD background was detected twice as often as during pregnancy prolongation (62.9% versus 30.4%, p = 0.03, χ2 = 4.9).

Recurrences of BV and microbial contamination of the cervical canal were indicators of an immunodeficiency state associated with the risk of RL in a sample with CP and anemia.

A change in the microbial community with the replacement of lactobacilli with Gardnerella vaginalis and anaerobic representatives acts as a trigger for an inflammatory reaction and an innate immune response of the vaginal epithelium.

In vitro studies have shown that G. vaginalis can weaken the barrier function of the epithelium through direct tissue damage and inflammation [29].

The high frequency of BV relapses is connected to the inability of antimicrobial drugs to eliminate vaginosis-associated infections [32]. BV can result in a 1.5-2 times increased risk of infection with C. trachomatis, N. gonorrhoeae, T. vaginalis [12]. The ascent of vaginosis-associated infections into the upper genital tract is accompanied by the probability of colonization of the placenta and the development of inflammatory diseases of the pelvic organs, infection of the fetus and placenta, and premature birth [28].

Simultaneous infection of the cervical canal and urine in pregnant women with anemia and CP indicates an initial decrease in nonspecific resistance, long-term persistence of pathogenic pathogens in the body of pregnant women and an increase in the frequency of intrauterine infection, which, in turn, causes adaptation breakdowns [2].

Interestingly, viable G. vaginalis bacteria can be absorbed by vaginal epithelial cells with the participation of active reorganization of the epithelial cytoskeleton, and that activates factors that promote the attachment of other pathogenic bacteria, for example, E. coli [23].

Microbial balance violations in the loci of the urogenital sphere are accompanied by changes in the immune system and homeostasis mechanisms, the deepening of which with the onset of pregnancy poses a threat of its termination.

The fact of a significant combination of infectious and inflammatory processes in the kidneys and vaginal dysbiosis in pregnant women with CP and anemia is consistent with the data on the violation of the vaginal biotope in 70-80% of pregnant women with CP [10,31].

The role of BV and persistent infectious and inflammatory process in the urinary tract as triggers of ovum infection is confirmed in the literature, along with the conclusion about the need for proper recovery of women before conceiving a child [3,5].

The analysis of the causes of suboptimal trophoblast invasion with a change in the immunomodulatory function of the placenta from the early stages of pregnancy made it possible to establish a number of regularities.

Abnormal vascular indices (pulsation index (PI) in the uterine arteries (> 1.5 at 6-8 weeks) ($\chi 2 = 9.1$, $p = 0.003$)) acted as markers of placental ischemia and were more informative in predicting miscarriage in combination with biochemical tests for evaluating placental function [18,19,30].

The co-factors of an early placental dysfunction associated with the probability of pregnancy termination were: low production of placental lactogen (<1.4 mg / ml) (min 1.5 times less than the initial) ($\chi 2 = 4.3$, $p = 0.04$), excessive PAPP-A (> 620 mU / l) (min 1.3 times more than the initial) ($\chi 2 = 8.4$, $p = 0.004$).

Obviously, chronic infectious and inflammatory processes in pregnant women with anemia and CP, especially on the background of changes in the microbiome of the urogenital tract, contribute to the abnormal development of the placenta with a violation in uterine-fetal interaction at the molecular-cellular level. The analysis of risk factors in women with EGD (CP and anemia) indicates a significant connection of the peculiarities of the course of pregnancy with the adaptive resources of the female body.

Pathomorphological examination of the abortion material (miscarriages and non-developing pregnancy) evacuated during uterine emptying of women with CP and anemia showed abnormal development of syncytiotrophoblast with tissue dystrophy, especially with leukocyte infiltration: samples with immaturity of villous trophoblast and endometritis / deciduitis (30.1%); incomplete gravidar transformation of the endometrium and inflammatory changes (22.4%); signs of endometritis in general (65.8%).

The persistence of the inflammatory process in the endometrium, which subsequently determines the violation of the uteroplacental-fetal hemodynamics during the waves of cytotrophoblast invasion, proves to be the basis for a comprehensive examination of women with pregnancy termination on the CP and anemia background in the first trimester.

Thus, pregnant women with anemia and CP are distinguished by dysregulation of the activity of the "placenta-fetus-kidney" complex associated with angiopathy of the uterine vessels [7,8], with aggravated disruption of homeostasis and the microbiome of the urogenital tract in the absence of pregravid recovery and / or prevention of early placental insufficiency due to the "crisis" of angiogenesis of the early placenta.

Reference

1. Воеводин С.М. Ультразвуковая и клинико-морфологическая оценка плацентарной дисфункции при критических состояниях у плода / Воеводин С.М., Шеманаева Т.В., Дубова Е.А. // Гинекология. –2013; 15(5): 65-69 [Voevodin SM. Shemanaeva TV, Dubova EA. Ultrasound and clinical and morphological assessment of placental dysfunction in critical conditions in the fetus. *Gynecology.* 2013; 15(5): 65-69 (In Russ.).]

2. Значимость диагностики инфекционной патологии в снижении репродуктивных потерь / Чижова Г.В. [и др.] //Проблемы стандартизации в здравоохранении. – 2010; 7:38-43 [Chizhova GV et al. The significance of the diagnosis of infectious pathology in reducing reproductive losses. *Problems of standardization in healthcare*. 2010; 7: 38-43 (In Russ.).]

3. Роль TLR и факторов мукозального иммунитета в патогенезе и предупреждении прерывания беременности при урогенитальной инфекции / Караулов А.В. [и др.] / Инфекционные болезни. – 2017; 15(4)82-90[Karaulov AV et al. The role of TLR and mucosal immunity factors in the pathogenesis and prevention of pregnancy termination in urogenital infection. *Infectious diseases*. 2017; 15(4): 82–90 (In Russ.).]

4. Охотникова Е.Н. Анемия при хронических заболеваниях / Охотникова Е.Н., Поночевная Е.В. // Клиническая иммунология. Аллергология. Инфектология. –2012; 5/6: 22-24 [Ohotnikova EN, Ponochevnaya EV. Anemia in chronic diseases. *Clinical immunology. Allergology. Infectology.* 2012; 5/6: 22-24 (In Russ.).]

5. Причины невынашивания беременности / Аполихина И.А., Шнейдерман М.Г., Тетерина Т.А., Горбунова Е.А. // Гинекология. –2013; 15(5):60-65 [Apolihina I.A., Schneiderman M.G., Teterina T.A., Gorbunova E.A. Causes of miscarriage. G*inekologiya*. 2013; 15(5): 60–65 (In Russ.).]

6. Радзинский В.Е. Биохимия плацентарной недостаточности. Радзинский В.Е., Смалько П.Я. –М.: Издательство РУДН. 2001. –275 с. [Radzinsky VE. Smalko PYa. Biochemistry of placental insufficiency. M: RUDN Publishing House. 2001; 275 (In Russ.).]

7. Радзинский В.Е. Акушерская агрессия. – М.: Status Praesens, 2018; 688 с. [Radzinsky V.E. Obstetric aggression. Moscow, Status Praesens. 2018; 688 (In Russ.).]

8. Радзинский ВЕ. Беременность ранних сроков. От прегравидарной подготовки к здоровой гестации / Радзинский ВЕ, Оразмурадов АА. – 3-е изд., испр. и доп. – М., Status Praesens. 2018; 800 с. [Radzinsky V.E., Orazmuradov A.A. Pregnancy of early terms. From pregravid preparation to healthy gestation, 3rd ed., Moscow., Status Praesens. 2018; 800 (In Russ.).]

9. Рымашевский А.Н. Перинатальная заболеваемость плодов и новорожденных женщин с хроническими инфекционно-воспалительными заболеваниями: автореф. дисс… д-ра. мед. наук. – М. 2006; 44 с. [Rymashevskiy A.N. Perinatal morbidity of fetuses and newborns in women with chronic infectious and inflammatory diseases. *Abstract of the dissertation of the candidate of biological sciences.* M: 2006; 44 (In Russ.).]

10. Широкова С.В. Характеристики влагалищного содержимого при различных экстрагенитальных заболеваниях беременных в ранние сроки гестации: автореф. дис. канд. биол., наук. –2008; 21 с. [Shirokova SV.Characteristics of the vaginal contents in various extragenital diseases of pregnant women in the early stages of gestation. *Abstract of the dissertation of the candidate. biological sciences*. 2008; 21 (In Russ.).]

11. Abdel-Raoufabdel-Aziz AR, Ali DK, Talkhan HM. Pregnancy outcome and the effect of maternal nutritional status. *J Egypt Soc Parasitol*. 2013; 43(1); P. 125-132.

12. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. Immunity. Anahtar MN, Byrne EH, Doherty KE. et al. 2015.19;42(5):965-76. doi: 10.1016/j.immuni.2015.04.019. PMID: 25992865; PMCID: PMC4461369.

13. Sun D, McLeod A, Gandhi S. et al. Anemia in Pregnancy: A Pragmatic Approach. *Obstet Gynecol Surv.* 2017;72(12):730-737.

14. Lai YJ, Hsu TY, Lan KC. et al. Asymptomatic pyuria in pregnant women during the first trimester is associated with an increased risk of adverse obstetrical outcomes. *Taiwan J Obstet Gynecol.* 2017;56(2):192-195.

15. Benirschke K, Burton GJ, Baergen RN. Pathology of The Human placenta. Sixth edition. Springer-Verlag Berlin Heidelberg. 2012.

16. Camaschella C. Iron-deficiency anemia. *N Engl J Med.* 2015; 372(19):1832-43. doi: 10.1056/NEJMra1401038. PMID: 25946282.

17. Cheesbrough M. District Laboatory Practice in Tropical Countries. Part 2. *Examinnation of urine*. United States of America by Cambridge University Press. New York: Cambridge university press; 2006: 105–15.

18. Mönckeberg M, Arias V, Fuenzalida R. et al. Diagnostic Performance of First Trimester Screening of Preeclampsia Based on Uterine Artery Pulsatility Index and Maternal Risk Factors in Routine Clinical Use. *Diagnostics (Basel).* 2020;10(4):182.

19. Arakaki T, Hasegawa J, Nakamura M. et al. First trimester measurements of the three-dimensional ultrasound placental volume and uterine artery Doppler in early- and late-onset fetal growth restriction / *Matern Fetal Neonatal Med.* 2018:1-181.

20. Glaser AP, Schaeffer A Urinary Tract Infection and Bacteriuria in Pregnancy *J. Urol Clin North Am*. 2015;42(4):547-60.

21. Influence of maternal anemia during pregnancy on placenta and newborns / Lelic M, Bogdanovic G, Ramic S. et al. *Med Arch.* 2014;68(3):184-7. doi: 10.5455/medarh.2014.68.184-187.

22. Iron deficiency in late pregnancy and its associations with birth outcomes in Chinese pregnant women: a retrospective cohort study / Yuan X, Hu H, Zhang M. et al. *Nutr Metab (Lond).* 2019;16:30. doi: 10.1186/s12986-019-0360-9. eCollection 2019.

23. Marrs CN, Knobel SM, Zhu WQ. Evidence for Gardnerella vaginalis uptake and internalization by squamous vaginal epithelial cells: implications for the pathogenesis of bacterial vaginosis. *Microbes Infect* 2012;14:500–508. doi:10.1016/j.micinf.2011.12.009.

24. Matuszkiewicz-Rowińska J, Małyszko J, Wieliczko M. Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problem. *Arch Med Sci.* 2015;11(1):67-77.

25. Milman N. Iron and pregnancy–a delicate balance. *Ann. Hematol 85*. 2006; 559–565. DOI: 10.1007/s00277-006-0108-2

26. Moreno Santillan AA, Briones Garduño JC, Diaz de Leon Ponce MA. Uric Acid in Pregnancy: New Concepts. *Contrib Nephrol*. 2018;192:110-115.

27. Nelson DB, Nelson DB, Bellamy S, Clothier BA. Characteristics and pregnancy outcomes of pregnant women asymptomatic for bacterial vaginosis. *Matern Child Health J*. 2008;12(2):216-22. doi: 10.1007/s10995-007-0239-7.

28. Onderdonk AB, Hecht JL, McElrath TF. Colonization of second-trimester placenta parenchyma. *Am J Obstet Gynecol*. 2008; 199:52.e1–52.e10. doi:10.1016/j.ajog.2007.11.068.

29. Patterson JL, Stull-Lane A, Girerd PH, Jefferson KK. Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of Gardnerella vaginalis relative to other bacterial-vaginosis-associated anaerobes Microbiology 2010; 156:392–399. doi:10.1099/mic.0.034280-0.

30. Rodriguez A, Tuuli MG, Odibo AO. First-, Second-, and Third-Trimester Screening for Preeclampsia and Intrauterine Growth Restriction Clin Lab Med. 2016;36(2):331-51.

31. Stapleton AE. The Vaginal Microbiota and Urinary Tract Infection. *Microbiol Spectr.* 2016;4(6).

32. Swidsinski A. Response of Gardnerella vaginalis biofilm to 5 days of moxifloxacin treatment. Swidsinski A, Dörffel Y, Loening-Baucke V. et al. *FEMS Immunol. Med. Microbiol*. 2011; 61, 41–46. 10.1111/j.1574-695X.2010.00743.x