THE RELATION BETWEEN FIRST TRIMESTER SCREENING MARKERS AND MATERNAL FOLIC ACID AND VITAMIN B12 LEVELS

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ABSTRACT

Objective: *To assess the relation between first trimester screening markers and maternal folic acid and vitamin B12 levels.*

Material and Method: *A retrospective study was conducted with 49 women of any age with a singleton pregnancy between 11-14 weeks of gestation who were offered routine first trimester prenatal screening and whose folic acid and vitamin B12 levels were measured during their first visit for any reason and who gave birth between September 2004 to May 2007 in the university hospital. Any possible correlation between these elements and screening markers were assessed. Pregnancies with male and female fetuses were compared.*

Results: *There were no patients with folic acid deficiency and in only 2 patients vitamin B12 level was low. Only one patient had an increased biochemical risk and all the patients gave birth to healthy babies. No significant correlation was found between assessed parameters when all patients were analyzed; however, when analysis was based on fetal gender it was found that maternal folic acid level was negatively correlated with PAPP-A levels in only pregnant women that gave birth to male fetuses (r: -0.376, p= 0.04). There was no significant difference in pregnancies with male and female fetuses with respect to assessed parameters.*

Conclusion: *There is a significant negative correlation between folic acid and PAPP-A in pregnancies with a male fetus, but not in pregnancies with a female fetus. The underlying mechanism is not clear and further studies are needed.*

Key Words: *hCH, PAPP-A, folic acid, vitamin B12, first trimester screening.* ***Nobel Med 2011; 7(2): 55-60***

BİRİNCİ TRİMESTER TARAMA BELİRTEÇLERİ İLE MATERNAL FOLİK ASİT VE VİTAMİN B12 SEVİYELERİ ARASINDAKİ İLİŞKİ

ÖZET

Amaç: Birinci trimester tarama belirteçleri ile maternal folik asit ve vitamin B12 seviyeleri arasındaki ilişkinin incelenmesi

Materyal ve Metod: Eylül 2004 ile Mayıs 2007 tarihleri arasında 11-14. gebelik haftası içinde olup kliniğimizde birinci trimester taraması yapılan, ilk vizitinde herhangi bir endikasyonla maternal folik asit ve vitamin B12 değerlerine bakılan ve doğumunu hastanemizde yapan toplam 49 gebe retrospektif olarak incelendi. Tarama belirteçleri ile folik asit ve vitamin B12 arasındaki ilişki korelasyon analizi ile incelendi. Erkek bebekli gebeliklerle kız bebekli gebelikler karşılaştırıldı.

Bulgular: Hiçbir gebede folik asit eksikliği yoktu ve 2 gebede vitamin B12 seviyesi düşüktü. Sadece bir hastada biyokimyasal risk artmış olarak bulundu ve bebeklerin hepsi sağlıklı olarak doğdular. Tüm gebelere bakıldığında folik asit ve vitamin B12 ile birinci trimester tarama belirteçleri arasında anlamlı ilişki bulunmadı; fakat analiz fetal cinsiyete göre yapıldığında erkek bebek doğuran gebelerde folik asit seviyesi ile PAPP-A seviyesi arasında anlamlı negatif bir korelasyon tespit edildi (r: -0,376, p= 0,04). Kız bebek doğuran gebelerde böyle bir ilişki yoktu (r= 0,047; p= 0,848). Erkek bebek ile kız bebek doğuran gebeler arasında incelenen parametreler bakımından herhangi anlamlı bir fark tespit edilmedi.

Sonuç: Erkek fetusa sahip olan gebelerde birinci trimesterde maternal folik asit seviyesi ile PAPP-A arasında anlamlı negatif bir korelasyon mevcuttur. Benzer bir ilişki kız fetusa sahip olan gebelerde mevcut değildir. Alta yatan mekanizma açık değildir ve ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: hCH, PAPP-A, folik asit, vitamin B12, birinci trimester taraması **Nobel Med 2011; 7(2): 55-60**

INTRODUCTION

Pregnancy is associated with cellular proliferation and increase in one-carbon metabolism[1,2] and folate and vitamin B12 are vital elements for cell division whose metabolism is intimately connected[3]. Folate plays a major coenzymatic role in nucleotide and amino acid synthesis and also in DNA methylation, all of which are important to the increased needs during pregnancy[4]. Vitamin B12 functions as a co-enzyme in the remethylation of homocysteine to methionine and in the conversion of methylmalonyl-CoA to succinyl-CoA[3]. Abnormally low circulating levels of these two elements have been shown to be associated with adverse pregnancy outcomes including spontaneous abortions, preeclampsia, low birth weight, vascular disorders of pregnancy or of the uteroplacental unit. [5-9]

Free beta human chorionic gonadotropin (fβhCG) and pregnancy associated plasma protein-A (PAAP-A) are mainly secreted by syncytiocytotrophoblasts of the placenta and have been used as markers of first trimester aneuploidy screening. From that point if folate and vitamin B12 affect placental function then these two elements may affect secretion of these two markers and affect results of first trimester screening.

The affect of folate and vitamin B 12 on first trimester screening markers has not been assessed. The aim of the present study was to investigate if there were any relation between maternal folate and vitamin B12 levels and first trimester screening markers.

MATERIAL and METHOD

A retrospective study was conducted with 49 women of any age with a singleton pregnancy between 11 and 14 weeks of gestation who were offered routine first trimester prenatal screening and whose folic acid and vitamin B12 levels were measured during their first visit for any reason and who gave birth between September 2004 to May 2007 in Gaziosmanpasa University Faculty of Medicine, Department of Obstetrics and Gynecology. Patients with multiple gestations, recent vaginal bleeding, known systemic diseases and pregnancies with fetal anomalies that were detected with sonography were excluded. All the data were obtained from patient files.

Prenatal screening was done on the basis of maternal age, levels of PAPP-A and fβ-hCG and measurement of NT. The screening policy of the hospital was explained in detail to the patients and both screening and diagnostic tests for the detection of fetal aneuploidy were discussed. All measurements were performed by experienced sonographers who were holders of The Fetal Medicine Foundation's Certificate of Competence for first trimester scanning using transabdominal ultrasound (Shimadzu SDU 1200 Tokyo, Japan). The NT was measured in the sagittal plane→

as the maximal sonolucent zone between the inner aspect of the fetal skin and the outer aspect of the cervical spine.

fβ-hCG and PAPP-A were measured using IMMULITE 1000 analyzer (BioDPC, USA). The concentrations of these markers were converted to MoMs (multiples of medians) using the relation with gestational age, corrected for maternal weight, ethnic origin and smoking status [10-13]. For screening a combined risk of ≥ 1/270 was assumed to be screen positive; however, for assessment of any possible relation between folic acid and vitamin B12 and fβ-hCG and PAPP-A biochemical screening risk calculations were also used. Again the cut-off level for being screen positive was assumed to be ≥ 1/270. Folic acid and vitamin B12 were measured by immunoassay method (Access® Immunoassay kits, Beckman Coulter Unicel DXI 800) during the first prenatal visit (7-14 weeks of gestation) for various indications including suspicion of absorption and consumption deficiencies. The laboratory reference values for folic acid and vitamin B12 were ≥3 pg/mL and 126.5-505 pg/mL respectively. Statistical analysis was accomplished on a personal computer by using statistical program for social sciences version 12.0 (SPSS 12.0, demo, SPSS Inc. Chicago, Illinois). Kolmogorov-Smirnov test with Lillefor's correction was used to test whether the variables used in the study were normally distributed. It was found that MoM values of fβ-hCG and PAPP-A were not normally distributed. Log10 transformation was applied to MoM values of fβ-hCG and PAPP-A and it was shown that both the transformed markers had normal distribution using Kolmogorov-Smirnov test. Independent samples t test was used for comparison of parametric data. For correlation analysis Pearson correlation coefficients were used. Statistical significance level was set at 5%.

RESULTS

The characteristics of the patients are depicted in Table 1. Before the present pregnancy 72.3% of the women had delivered before and 27.7% were nulliparous. 6.1% of the patients were smokers. 61.2% of the fetuses (30/49) were male and 38.8% (19/49) were female. No women had folic acid level <3 pg/mL and 2 patients (4.1%) had vitamin B12 levels <126.5 pg/mL.

Correlation of maternal serum folic acid, vitamin B12 and first trimester screening markers are shown in Table 2. No significant relation was found between the assessed parameters; however, when this analysis was performed with respect to fetal gender, it was found that there was a statistically significant negative correlation between maternal folic acid and PAPP-A levels in male fetuses (r= -0.376; p= 0.040) (Table 3) (Figure 1). Such an association was not found in female fetuses (r= 0.047; p= 0.848). Table 4 shows the comparison of assessed parameters between male and female fetuses. There were no significant differences in any of the assessed parameters.

Only one pregnant woman among 49 women had an increased biochemical screening risk and none of the women had increased combined risk in screening. There were no malformations in any of the 49 fetuses.→

Table 1: Patient characteristics

	Mean ± SD
Age (years)	29.06 ± 4.15
Weight (kg)	65.53 ± 10.79
Gestational week	12.11 ± 0.73
CRL(mm)	58.98 ± 9.94
NT (MoM)	0.99 ± 0.23
Fetal Birth Weight (g)	3414.89 ± 451.32
fβ-hCG (Log10 MoM)	-0.102 ±0.261
PAPP-A (Log10 MoM)	-0.019 ± 0.245
Vitamin B12 (pg/mL)	226.61 ± 84.56
Folic acid (pg/mL)	10.56 ± 4.58

CRL: Crown–rump length, **PAPP-A:** pregnancy associated plasma protein-A, **fβ-hCG:** free beta human chorionic gonadotropin, **NT:** nuchal translucency

Table 2: Correlation of first trimester markers, folic acid and vitamin B12.

	Folic acid (pg/mL)		**Vitamin B12 (pg/mL)**	
	r	**p**	**r**	**p**
PAPP-A (Log 10 MoM)	-0.161	0.268	0.103	0.481
fβ-hCG (Log 10 MoM)	0.142	0.330	0.055	0.709
NT MoM	0.020	0.891	0.073	0.623

PAPP-A: pregnancy associated plasma protein-A, **fβ-hCG:** free beta human chorionic gonadotropin, **NT:** nuchal translucency

Table 3: Correlation of first trimester markers, folic acid and vitamin B12 in male and female fetuses

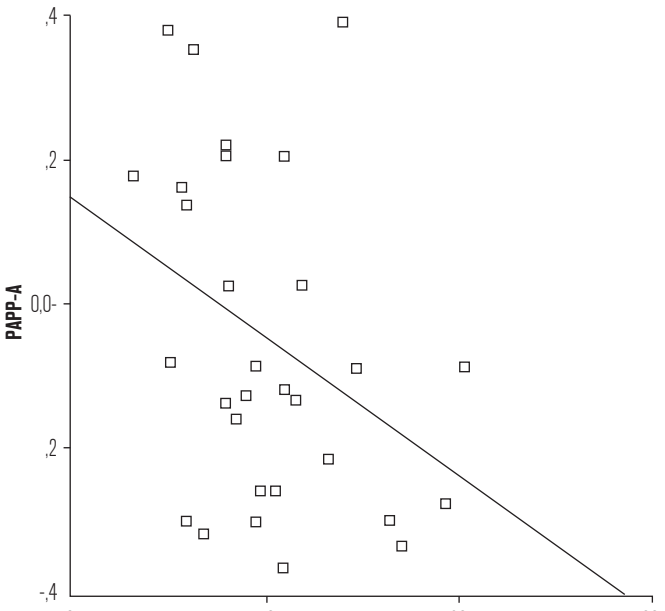
	Folic acid (pg/mL)		**Vitamin B12 (pg/mL)**	
Male	**r**	**p**	**r**	**p**
PAPP-A (Log 10 MoM)	-0.376	0.040*	0.161	0.396
fβ-hCG (Log 10 MoM)	0.850	0.655	0.171	0.368
NT MoM	0.194	0.313	0.313	0.098
Female	**r**	**p**	**r**	**p**
PAPP-A (Log 10 MoM)	0.047	0.848	-0.040	0.871
fβ-hCG (Log 10 MoM)	0.197	0.418	-0.365	0.124
NT MoM	-0.135	0.581	-0.351	0.140

*:Istatistically significant, **PAPP-A:** pregnancy associated plasma protein-A, **fβ-hCG:** free beta human chorionic gonadotropin, **NT:** nuchal translucency

Table 4: Comparison of parameters between pregnancies with male and female fetuses

	Male Fetus	Female Fetus	p
Age (years)	29.99 ± 4.29	27.59 ± 3.54	0.480
Weight (kg)	67.07 ± 11.52	63.11 ± 9.31	0.214
Gestational week	12.00 ± 0.68	12.29 ± 0.78	0.179
CRL(mm)	59.59 ± 9.49	61.11 ± 10.47	0.234
NT (MoM)	0.97 ± 0.18	1.04 ± 0.29	0.332
Fetal Birth Weight (g)	3474.67 ± 515.76	3320.53 ± 315.04	0.248
fβ-hCG (Log10 MoM)	-0.157 ± 0.25	-0.015 ± 0.26	0.062
PAPP-A (Log10 MoM)	-0.041 ± 0.22	0.014 ± 0.28	0.452
Vitamin B12 (pg/mL)	217.89 ± 97.39	240.37 ± 58.78	0.370
Folic acid (pg/mL)	49.48 ± 1.79	48.89 ± 2.11	0.301

CRL: Crown–rump length, **PAPP-A:** pregnancy associated plasma protein-A, **fβ-hCG:** free beta human chorionic gonadotropin, **NT:** nuchal translucency



r=-0.376, p=0.04; Pearson correlation analysis

Figure 1. Correlation between maternal folic acid and PAPP-A in male fetuses.

DISCUSSION

To our knowledge, this is the first study that assessed any possible relation between maternal serum folic acid and vitamin B12 levels and first trimester screening markers. In overall no relationship was found; however, when analysis was based on fetal gender it was found that maternal folic acid level was negatively correlated with PAPP-A levels in only pregnant women that gave birth to male fetuses.

Vitamin B12 is an essential nutrient in humans and in pregnancy a progressive reduction of this vitamin is documented[1,14]. Deficiency of this vitamin causes megaloblastic anemia, neuropsychiatric symptoms, severe retardation of fetal nervous system myelination, spontaneous abortions and may be involved in neural tube defect etiology[6,15-19]. Folic acid deficiency also has been reported to be associated with neural tube defects, other malformations[20], and common pregnancy complications including preterm delivery, low birth weight, preeclampsia and spontaneous abortions[21-23]. Steengers-Theunissen et al reported that folate deficiency, at the level of cytotrophoblast cells, induces apoptosis [24].

Folate and vitamin B12 are closely related with homocysteine. 5-methyltetrahydrofolate and vitamin B12, as methylcobalamin, are essential for the functioning of the enzyme methionine synthase that is involved in the re-methylation of homocysteine into methionine[25]. As a consequence deficiency of both folate and cobalamin leads to elevated total homocysteine level in plasma or serum[25,26]. The relation between elevated homocysteine and obstetric complications that are connected with vascular disorders of pregnancy or of uteroplacental unit has been shown before [27-30]. Di Simone et al reported that human placenta is a target for homocysteine and in trophoblast cells exposed to homocysteine cellular apoptosis and the inhibition of trophoblast functions have been observed [31,32]. In another study conducted by the same investigators in which effect of folic acid on homocysteine induced trophoblast apoptosis was studied, it was found that homocysteine induced DNA fragmentation and significantly reduced hCG secretion and addition of folic acid resulted in inhibition of the effects of homocysteine on human trophoblast [32].

One would expect a positive, if any, correlation between maternal folate or vitamin B12 and fβhCG and PAPP-A secretion from the placenta as these two vitamins either directly or by homocysteine route affect function of trophoblasts. Di Simone et al reported a relation between folic acid and hCG secretion [32]. Relation between folate or vitamin B12 and PAPP-A has not been assessed before. In the present study no association was found between folic acid, vitamin B12 and assessed screening markers when all 49 patients were analyzed. The possible explanation would be that in the present study no women had folic acid levels less than the normal laboratory cut-off value and only two women had low vitamin B12 levels (118.7 and 47.13 pg/mL). Because folic acid and vitamin B12 levels were in normal range, homocysteine metabolism would not be disturbed. From this point we can conclude that normal levels of folic acid and vitamin B12 do not seem to affect secretion of fβ-hCG and PAPP-A when fetal gender was ignored. However when fetal gender was considered, although the folic acid levels were within normal ranges it was found that→

in pregnancies with male fetuses contrary to what one might expect, an inverse relation was found between folic acid and PAPP-A. Such an association was not found in pregnancies with a female fetus. The possible mechanism underlying this significant inverse relation is not clear.

PAPP-A has been identified as a metalloprotease cleaving insulin-like growth factor binding protein-4 (IGFBP-4) and seems to be an important regulator of local IGF bioavailability and cell growth [33,34]. In human reduced levels are associated with several situations including Cornelia Delange syndrome, intrauterine growth retardation, extremely premature delivery and fetal chromosomal aneuploidies [35,36]. This decrease in PAPP-A has been suggested to result from defective syncytiotrophoblast formation and function [37]. Folic acid has been reported to inhibit cytotrophoblast apoptosis and resolve the inhibitory effect of homocysteine on human trophoblast [24,32]. From this data a positive relation or no relation could be expected because if folic acid had a direct affect on cytotrohoblasts then it should be noted that PAPP-A is secreted by syncytiotrophoblasts and secondly if folic acid has any affect indirectly by homocysteine metabolism the level of folic acid is in normal range so that homocysteine is not in excess. However an inverse relation and only in pregnancies with a male fetus is not expected. In the present study no significant difference was found between PAPP-A levels in pregnancies with male and female fetuses. Further studies including patients with folic acid and or vitamin B12 deficiencies and measured homocysteine levels are necessary to clarify this issue. There are some limitations in the present study. This preliminary study is a retrospective study with a small sample size. There were only two women with low vitamin B12 level and no women with low levels of folic acid. Larger studies including patients with abnormal levels of these vitamins are necessary.

In conclusion in the present study, for the first time to our knowledge any possible relation between maternal serum folic acid and vitamin B12 levels and first trimester screening markers was assessed and it was found that there was no relation between these vital elements and fβ-hCG secretion and NT measurement; however, there is a significant negative correlation between folic acid and PAPP-A in pregnancies with a male fetus, but not in pregnancies with a female fetus. The underlying mechanism is not clear and further studies are needed.

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REFERENCES

1. Bruinse HW, van den Berg H. Changes of some vitamin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1995; 61: 31– 37.
2. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr 2006; 83: 993–1016
3. Guerra-Shinohara EM, Paiva AA, Rondo PH, et al. Relationship between total homocysteine and folate levels in pregnant women and their newborn babies according to maternal serum levels of vitamin B12. BJOG 2002; 109: 784- 791.
4. Oommen AM, Griffin JB, Sarath G, Zempleni J. Roles of nutrients in epigenetic events. J Nutr Biochem 2005; 16: 74–77.
5. Vander Molen EF, Verbruggen B, Novakova I, et al. Hyperhomocysteinaemia and other thrombotic risk factors in women with placental vasculopathy, Br J Obstet Gynaecol 2000; 107: 785–791.
6. Adams MJ Jr, Khoury MJ, Scanlon KS, et al. Elevated midtrimester serum methylmalonic acid levels as a risk factor for neural tube defects. Teratology 1995; 51: 311-317
7. Wilson A, Platt R, Wu Q, et al. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. Mol Genet Metab 1999; 67: 317- 323.
8. Ray JG, Laskin CA. Folic acid and homocysteine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systematic review. Placenta 1999; 20: 519-529
9. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr 2000; 71: 962-968.
10. Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. BJOG 2000; 107: 1265-1270
11. Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. Prenat Diagn 2004; 24: 169-173.
12. Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. Prenat Diagn 2003; 23: 851-855.
13. Spencer K, Heath V, El-Sheikhah A, Ong CY, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. Prenat Diagn 2005; 25: 365-369.
14. Cikot RJ, Steegers-Theunissen RP, Thomas CM, et al. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001; 85: 49-58.
15. Lövblad K, Ramelli G, Remonda L, et al. Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatr Radiol 1997; 27: 155-158.
16. Savage DG, Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. Baillieres Clin Haematol 1995; 8: 657-678.
17. Monagle PT, Tauro GP. Infantile megaloblastosis secondary to maternal vitamin B12 deficiency. Clin Lab Haematol 1997; 19: 23-25.
18. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988; 318: 1720-1728.
19. Rowland AS, Baird DD, Shore DL, et al. Nitrous oxide and spontaneous

abortion in female dental assistants. Am J Epidemiol 1995; 141: 531-538.
20. Allen WP. Folic acid in the prevention of birth defects. Curr Opin Pediatr 1996; 8: 630-634.
21. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996; 63: 520-525.
22. Leeda M, Riyazi N, de Vries JI, et al. Effects of folic acid and vitamin B6 supplementation on women with hyperhomocysteinemia and a history of preeclampsia or fetal growth restriction. Am J Obstet Gynecol 1998; 179: 135-139.
23. Nelen WL, Blom HJ, Steegers EA, et al. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000; 95: 519-524.
24. Steegers-Theunissen RP, Smith SC, Steegers EA, Guilbert LJ, Baker PN. Folate affects apoptosis in human trophoblastic cells. BJOG 2000; 107: 1513-1513.
25. Brouns R, Ursem N, Lindemans J, et al. Polymorphisms in genes related to folate and cobalamin metabolism and the associations with complex birth defects. Prenat Diagn 2008; 28: 485-493
26. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. FASEB J 1993; 7: 1344-1353.
27. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 1992; 69: 915-926.
28. Powers RW, Evans RW, Majors AK, et al. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. Am J Obstet Gynecol 1998; 179: 1605-1611.
29. Buemi M, Marino D, Di Pasquale G, et al. Effects of homocysteine on proliferation, necrosis, and apoptosis of vascular smooth muscle cells in culture and influence of folic acid. Thromb Res 2001; 104: 207-213.
30. Khong TY, Hague WM. The placenta in maternal hyperhomocysteinaemia. Br J Obstet Gynaecol 1999; 106: 273-278.
31. Di Simone N, Maggiano N, Caliandro D, et al. Homocysteine induces trophoblast cell death with apoptotic features. Biol Reprod 2003; 69: 1129-1134.
32. Di Simone N, Riccardi P, Maggiano N, et al. Effect of folic acid on homocysteine-induced trophoblast apoptosis. Mol Hum Reprod 2004; 10: 665-669.
33. Lawrence JB, Oxvig C, Overgaard MT, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci USA 1999; 96: 3149-3153
34. Giudice LC, Conover CA, Bale L, et al. Identification and regulation of GFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. J Clin Endocrinol Metab 2002; 87: 2359–2366.
35. Smith GC, Stenhouse EJ, Crossley JA, et al. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab 2002; 87: 1762–1767.
36. Spencer K, Aitken DA, Crossley JA, et al. First trimester biochemical screening for trisomy 21: the role of free β hCG, a fetoprotein and pregnancy associated plasma protein A. Ann Clin Biochem 1994; 31: 447–454.
37. Guibourdenche J, Frendo JL, Pidoux G, et al. Expression of pregnancy-associated plasma protein-A (PAPP-A) during human villous trophoblast differentiation in vitro. Placenta 2003; 24: 532-539.