

# The relationship between macro- and micro-nutrients intake and risk of preterm premature rupture of membranes in pregnant women of Isfahan

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## Abstract

**Background:** Since preterm premature rupture of membranes (PPROM) is one of the most important complications of pregnancy and its relationship with nutrition status have not been surveyed comprehensively, we decided to study the relationship of maternal received nutrients (36 macro- and micro-nutrients) in three trimesters and PPRM which could be considered as a unique study.

**Materials and Methods:** In this prospective cohort study, data was collected by filling a questionnaire through interviews with 620 pregnant women who had no parameters to affect pregnancy outcome. 48-hr dietary recalls were completed for eligible women at 11<sup>th</sup>–15<sup>th</sup>, 26<sup>th</sup>, 34<sup>th</sup>–37<sup>th</sup> weeks of gestation. Physical activity was also assessed using a standard questionnaire. Also pregnant mother's reproductive and demographic characteristic and supplementation are considered.

**Results:** The mean value of received saturated fatty acids, polyunsaturated fatty acids and energy, in the first trimester ( $P < 0.001$ ,  $P = 0.007$ , and  $P < 0.001$  respectively), the mean values of calcium, sodium intake in the second trimester ( $P = 0.045$ ,  $P = 0.006$ , and  $P = 0.004$  respectively), Vitamins C, A (mg),  $\beta$ -carotene, carotenoids intake in the second trimester ( $P = 0.03$ ,  $P = 0.001$ ,  $P = 0.007$ , and  $P = 0.01$  respectively), and higher Vitamin C intake during the first trimester ( $P = 0.02$ ) was significantly greater among subjects with PPRM compared to the others.

**Conclusions:** The mean value of mentioned received nutrients in subjects who experienced PPRM later in pregnancy was higher than the others, which is independent of demographic and reproductive characteristic, estimated physical activity, and supplementation. Therefore, these findings could be considered in the nutritional programming for pregnant women to manage the risk of PPRM.

**Key Words:** Macro nutrients, minerals, pregnancy outcome, preterm premature rupture of membranes, vitamins

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## INTRODUCTION

Preterm premature rupture of membranes (PPROM)

is defined as spontaneous rupture of the membranes at <37 weeks gestation at least 1 hour before the onset

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of contractions. It precedes delivery in approximately 25–30% of patients delivering prematurely.<sup>[1]</sup> Up to 4% of all pregnancies are complicated by PPRM.<sup>[2]</sup> It is associated with considerable increase in adverse maternal, fetal and neonatal risk.<sup>[3]</sup> The etiology of PPRM is believed to be multifactorial.<sup>[4]</sup> Maintenance of the chorioamniotic sac throughout normal pregnancy requires a balance between collagen synthesis by fibroblasts and collagenolytic activity by controlled responses of enzymes expressed in the fetal membranes or derived from inflammatory cells.<sup>[5,6]</sup> Reactive oxygen species (ROS) could be responsible for collagen damage in the chorioamniotic sac leading to tearing. Normally a balance exists between production and elimination of ROS. Oxidative stress (OS) occurs when pro-oxidants exceed antioxidants.<sup>[7-9]</sup> OS caused by increased ROS formation may modify the strength and the elasticity of collagen and cause PROM.<sup>[5,10]</sup> Therefore, PPRM may be an effect of collagen damage caused by increased ROS formation and/or antioxidant deletion, too.<sup>[10,11]</sup>

Micronutrient deficiencies that affect collagen formation have been shown to alter collagen structure and this has been associated with an increased risk of PPRM.<sup>[4,12,13]</sup> For instance, an association between low Vitamin C levels and PPRM has been reported.<sup>[14]</sup> Ferguson *et al.* looked at the association of PPRM to poor nutritional status using biochemical markers and dietary intake of micronutrients for the 1<sup>st</sup> time.<sup>[15]</sup>

On the other hand, Vitamins C and E supplementation at the recommended doses has been reported with an increased risk of PROM and PPRM.<sup>[16,17]</sup> Thus, we decided to survey the association between nutrition status of pregnant women and PPRM, especially it was urged by some of Cochrane reviews on this issue.<sup>[18]</sup> Nutritional status during pregnancy can be described by indicators of body size such as body mass index (BMI), nutritional intake, and serum assessments for various serum analytes.<sup>[4]</sup> In this study, the association between PPRM risk and intake of macro- and micro-nutrients during the first, second, and third trimesters was assessed. Our hypothesis is that the macro- and micro-nutrients are associated with PPRM.

## MATERIALS AND METHODS

This prospective cohort study (approved by the Institutional Review Board of Isfahan University of Medical Sciences) was conducted in four stages (The first, second, and third trimester of pregnancy and postpartum) on a group of 620 Iranian pregnant women aged 15–49 years between 10 and 40 gestational weeks whose delivery in hospital led to the birth of alive and apparently healthy baby. In addition,

they referred to 18 health centres and 12 private offices from April 2009 to August 2010. Considering the fact that 65% of women in Isfahan refer to health centres, 20% to private offices, and 15% to both, convenient sampling was performed. The women were in good general health, with no history of prior adverse pregnancy outcome. Exclusion criteria were conditions or factors causing PPRM, preterm delivery, low birth weight, and preeclampsia (placenta previa, placenta abruption, diabetes mellitus, gestational diabetes, uterine abnormalities, twine or multiple pregnancies, cervix incompetence, oligo-hydramnios, poly-hydramnios, <2 years pregnancies interval, trauma or surgery in the present pregnancy, urinary tract, genital system infections, abnormal presentations, and still birth). Other factors included smoking and drug addiction, digestive and metabolic diseases, hyper emesis gravidarum, gastro intestinal diseases, active hepatitis and hepatic disorders, hemoglobinopathies and anemia's, eating disorders, allergies, mental diseases and neurologic disorders, malignancy, Gaucher's diseases, chronic inflammatory diseases, asthma and respiratory system diseases, and cardiovascular diseases.<sup>[19-21]</sup> Taking their consent, data was collected by a questionnaire which was completed through interviews with pregnant women and prenatal and obstetric care-related records considering demographic and reproductive characteristics and physical activity as important confounding variables. In addition to this, 48-h dietary recalls were completed for eligible pregnant women at the 11<sup>th</sup>–15<sup>th</sup>, 26<sup>th</sup>, and 34<sup>th</sup>–37<sup>th</sup> weeks of gestation.<sup>[22-24]</sup> Physical activity was considered as any physical movement due to skeletal muscles resulting in energy consumption. Data on physical activity was collected using a standard pregnancy physical activity questionnaire which consists of 4 parts including physical activity at home, exercise, leisure activities, and workplace activity.<sup>[25]</sup> Therefore, the performed physical activities within 48 h were also assessed at the 11<sup>th</sup>–15<sup>th</sup>, 26<sup>th</sup>, and 34<sup>th</sup>–37<sup>th</sup> weeks of gestation (Physical activity was measured in metabolic equivalent of task-hours [MET-hours] of each activity multiplied by the duration of the activity in the day. MET-hours is a unit for estimating the metabolic cost or oxygen consumption of a particular physical activity, according to a standard questionnaire). Total amount of activity was calculated by summing the activities in the 3 trimesters and was used for analysis. The subjects were then followed until the end of pregnancy. The risk of PPRM was collected through patient records. To increase reliability, all interviewers were trained in the same conditions. On the other hand, patient records were completed by experts who guaranteed their reliability. In addition, since the records are prepared in fixed and standardized forms,

their reliability confidence has already been proven. Data obtained from the 48-hr dietary recalls was analyzed using Nutrition-IV software. Data analysis was performed in SPSS 18.0 (IBM Company, the United States) using *t*-test, binary logistic regression, and Chi-square analysis. *P* < 0.05 were considered significant in all tests.

Kolmogorov–Smirnov test was used to determine if the data followed Gaussian distribution. It has been shown that the distribution of macro- and micro-nutrients intake variables followed Gaussian distribution in groups with and without PPRM. So Student’s *t*-test was used to compare the received macro- and micro-nutrients means between two groups.

## RESULTS

According to our findings, 17 patients out of the 620 studied pregnant women (2.74%) were diagnosed with PPRM. Their demographic and reproductive characteristics are shown in Table 1.

None of personal and reproductive characteristics which are shown in Table 1 weren’t significantly associated with PPRM.

Mean values of micro- and macro-nutrient were calculated in the first, second and third trimesters [Table 2a-e]. The mean of received saturated fat ( $26.20 \pm 14.35$  in compare of  $68.96 \pm 154.58$ , *P* < 0.001), polyunsaturated fat ( $33.77 \pm 11.82$  in compare of  $41.77 \pm 15.71$ , *P* = 0.007) and energy ( $2299.59 \pm 729.57$  in compare of  $3134.75 \pm 2234.06$ , *P* < 0.001), in the second trimester in subjects who experienced PPRM later in pregnancy was higher than the other pregnant women. In addition, the mean values of iron ( $20.47 \pm 8.79$  in compare of  $24.87 \pm 11.59$ , *P* = 0.04), calcium ( $943.26 \pm 425.01$  in compare of  $1234.20 \pm 426.14$ , *P* = 0.006), and sodium ( $3080.73 \pm 1621.73$  in compare of  $4252.58 \pm 2845.20$ , *P* = 0.004) intake in the second trimester among women with PPRM were significantly more than healthy pregnant women. Vitamins C ( $152.70 \pm 105.76$  in compare of  $208.66 \pm 193.10$ , *P* = 0.03), A (mg) ( $780.95 \pm 1067.45$  in compare of  $1707.11 \pm 2929.92$ , *P* = 0.001), β-carotene ( $371.80 \pm 569.39$  in compare of  $783.31 \pm 525.75$ , *P* = 0.007), carotenoids ( $371.80 \pm 569.39$  in compare of  $783.32 \pm 1975.79$ , *P* = 0.01) intake in the second trimester was significantly greater among subjects with PPRM compared to the others.

Also significant relation between higher Vitamin C intake during the first trimester and PPRM risk was found ( $147.90 \pm 99.84$  in compare of  $206.23 \pm 156.47$ , *P* = 0.02).

**Table 1: Demographic and reproductive characteristic of the participants**

Maternal characteristics	PPROM		No PPRM		<i>P</i>
	Mean±SD	<i>n</i>	Mean±SD	<i>n</i>	
Age (year)	25.88±5.10	17	25.63±4.43	596	0.81
Gestational age (week)	36.21±2.71	17	38.76±1.48	597	<0.001
Total weight gaining during pregnancy (kg)	11.39±3.36	17	12.07±4.17	630	0.43
Income (thousands Rial)	246.65±132.2	16	282.195±114.3	585	0.22
Number of children	2.76±0.97	17	2.57±0.91	596	0.39
Mothers BMI before pregnancy (kg)	22.39±2.64	17	23.62±3.92	641	0.07
Weight gaining (first trimester) (kg)	1.51±1.37	18	1.66±2.01	636	0.20
Weight gaining (second trimester) (kg)	4.84±2.33	18	5.55±2.50	649	0.77
Weight gaining (third trimester) (kg)	5.62±3.1	15	5.06±2.6	630	0.12

SD: Standard deviation, BMI: Body mass index, PPRM: Preterm premature rupture of membranes

**Table 2a: Maternal intake of macronutrients in the first, second, and third trimesters of pregnancy**

Nutrients (trimesters)	Mean±SD		<i>P</i>
	No PPRM (n=570)	PPROM (n=17)	
Sugar (the first trimester)	89.78±49.70	111.35±55.80	0.07
Sugar (the second trimester)	100.12±50.66	116.41±61.96	0.19
Sugar (the third trimester)	102.29±49.37	95.50±46.65	0.66
Fiber (the first trimester)	20.37±10.49	22.27±7.10	0.45
Fiber (the second trimester)	23.58±12.13	26.41±9.86	0.34
Fiber (the third trimester)	24.01±11.87	21.73±6.09	0.54
Carbohydrate (the first trimester)	298.96±125.31	338.30±102.94	0.20
Carbohydrate (the second trimester)	341.19±113.10	390.62±135.70	0.08
Carbohydrate (the third trimester)	340.91±100.35	353.09±112.20	0.70
Protein (the first trimester)	92.61±37.12	106.36±37.11	0.13
Protein (the second trimester)	103.04±36.35	119.04±33.17	0.07
Protein (the third trimester)	106.44±81.33	96.29±25.30	0.69
Energy (the first trimester)	2024.31±775.67	2244.05±723.52	0.24
Energy (the second trimester)	2299.59±729.57	3134.75±2234.06	0.000**
Energy (the third trimester)	2378.72±1347.38	2329.75±650.96	0.98

\*\*Statistically significant. SD: Standard deviation, PPRM: Preterm premature rupture of membranes

Except for the mentioned variables, there were no significant differences between other micro- and macro-nutrients and the risk of PPRM [Table 2a-e].

In this study, all variables associated with PPRM were also analyzed by logistic regression analysis. This analysis method showed that these variables could predict PPRM affection in 97.4% of the cases. The

**Table 2b: Maternal Intake of fatty acids and cholesterol in the first, second, and third trimesters of pregnancy**

Nutrients (trimesters)	Mean±SD		P
	No PPRM (n=570)	PPROM (n=17)	
SFA (the first trimester)	22.50±12.65	23.53±12.94	0.74
SFA (the second trimester)**	26.20±14.35	68.96±154.58	0.000**
SFA (the third trimester)	26.86±43.91	26.43±11.57	0.97
MUFA (the first trimester)	25.59±17.00	26.50±00.00	0.82
MUFA (the second trimester)**	28.99±18.56	50.00±70.35	0.000**
MUFA (the third trimester)	28.75±42.09	24.00±10.70	0.72
DHA (the first trimester)	0.62±0.53	0.71±0.56	0.51
DHA (the second trimester)	0.69±0.55	0.71±0.35	0.86
DHA (the third trimester)	0.80±1.186	0.51±0.28	0.62
EPA (the first trimester)	0.62±0.53	0.71±0.56	0.48
EPA (the second trimester)	0.69±0.55	0.71±0.35	0.82
EPA (the third trimester)	0.79±1.86	0.51±0.28	0.62
Linolenic acid (the first trimester)	0.94±5.80	0.79±0.58	0.91
Linolenic acid (the second trimester)	1.03±5.82	0.92±0.36	0.93
Linolenic acid (the third trimester)	1.09±4.94	0.68±0.44	0.79
Linoleic acid (the first trimester)	12.29±11.62	12.35±6.51	0.98
Linoleic acid (the second trimester)	13.53±11.77	15.96±7.14	0.39
Linoleic acid (the third trimester)	13.94±12.00	16.32±5.76	0.53
PUFA (the first trimester)	30.56±13.03	34.36±11.66	0.23
PUFA (the second trimester)**	33.77±11.82	41.77±15.71	0.007**
PUFA (the third trimester)	33.89±17.23	34.90±8.70	0.85
Oleic (the first trimester)	22.12±10.95	26.00±11.27	0.15
Oleic (the second trimester)	25.44±11.15	28.94±13.28	0.20
Oleic (the third trimester)	24.36±11.20	23.10±9.63	0.91
Cholesterol (the first trimester)	234.04±202.60	196.17±85.58	0.42
Cholesterol (the second trimester)	257.66±136.76	267.24±130.35	0.76
Cholesterol (the third trimester)	266.46±337.10	265.85±186.20	0.99

\*\*Statistically significant. SD: Standard deviation, PPRM: Preterm premature rupture of membranes, SFA: Saturated fatty acid, PUFA: Poly unsaturated fatty acid, MUFA: Most unsaturated fatty acid, DHA: Docosahexanoic acid, EPA: Eicosapentanoic acid

most significant risk factor for PPRM was Vitamin C intake in the first trimester. Others have been shown respectively in Table 3 (The more Wald statistic, the stronger significant relationship).

Based on the calculated values of physical activity per day, no significant relations were found between physical activity during the first, second, and third trimesters and risk of PPRM [Table 4].

## DISCUSSION

In this prospective cohort study of pregnancies, we found increased risk for PPRM among women reporting higher dietary intake of saturated fatty acids (SFA), polyunsaturated fatty acids (PUFAs), energy, iron, calcium, sodium, Vitamins C, A,  $\beta$ -carotene, Carotenoid, in the second trimester (26<sup>th</sup> weeks of gestation) and Vitamin C intake in the first trimester (11–15<sup>th</sup> week of gestation).

Women with normal pregnancies experience increased OS compared with nonpregnant women. Elevations appear by the second trimester and gradually diminish

later in gestation, decreasing further after delivery.<sup>[26]</sup> Overproduction or inadequate control of OS may be involved in the etiology of obstetric complications such as PPRM.<sup>[27]</sup> Macronutrients are either targets of oxidative modifications after absorption or are present in a pro-oxidant form in the diet.<sup>[28]</sup> For instance, the effect of unsaturated fat intake on lipoprotein oxidation and lipoprotein oxidizability are documented along with the widely accepted increased antioxidant demand associated with the intake of diets rich in PUFA.<sup>[29]</sup>

According to our results, the mean of saturated fat intake during the second trimester in pregnant women who developed PPRM in later weeks of pregnancy was more than the other subjects ( $P < 0.001$ ). Detrimental effects attributable to saturated fat consumption have been shown by increased inflammatory bioactivity,<sup>[30]</sup> increased lipid per oxidation,<sup>[31]</sup> decreased antioxidant activity,<sup>[32]</sup> and enhanced cytokine levels<sup>[33]</sup> which can be involved in the pathophysiology of PPRM.

Our data showed that higher intake of poly unsaturated fat (both omega-3 and omega-6) is associated with the risk of PPRM ( $P = 0.007$ ).

Although there seems to be a general agreement that moderate intake of long-chain PUFAs (LCPUFA) reduce the risk of some diseases, there are indications that too high intake of polyunsaturated fat may be harmful. These harmful effects ascribed to their pro-oxidative role.<sup>[22,32]</sup>

**Table 2c: Maternal intake of micronutrients (vitamins) in the first, second, and third trimesters of pregnancy**

Nutrients (trimesters)	Mean±SD		P
	No PPROM (n=570)	PPROM (n=17)	
D (the first trimester)	2.86±3.72	2.67±2.61	0.83
D (the second trimester)	3.04±3.31	4.07±3.92	0.20
D (the third trimester)	3.24±3.63	3.33±3.74	0.93
B12 (the first trimester)	3.88±8.02	5.14±10.62	0.52
B12 (the second trimester)	4.67±9.64	3.34±1.48	0.57
B12 (the third trimester)	5.05±15.27	2.61±1.71	0.61
Fol (the first trimester)	288.39±179.75	285.67±151.68	0.95
Fol (the second trimester)	304.70±133.99	325.94±98.51	0.51
Fol (the third trimester)	326.78±177.94	279.03±89.66	0.39
B6 (the first trimester)	1.42±0.79	1.71±0.83	0.13
B6 (the second trimester)	1.45±0.69	1.63±0.70	0.31
B6 (the third trimester)	1.50±0.97	1.20±0.52	0.33
B3 (the first trimester)	20.01±9.00	22.06±6.04	0.35
B3 (the second trimester)	22.78±9.16	24.90±6.59	0.34
B3 (the third trimester)	23.87±25.80	22.10±5.58	0.82
B2 (the first trimester)	1.53±0.73	1.69±0.68	0.40
B2 (the second trimester)	1.69±0.90	1.80±0.53	0.61
B2 (the third trimester)	1.68±1.25	1.51±0.44	0.66
B1 (the first trimester)	1.60±0.69	1.92±0.71	0.06
B1 (the second trimester)	1.76±0.66	1.93±0.53	0.30
B1 (the third trimester)	1.76±0.74	1.78±0.57	0.95

\*\*Statistically significant. SD: Standard deviation, PPROM: Preterm premature rupture of membranes

Nutrients like LCPUFA are precursors of important bioactive compounds such as the prostacyclins, prostaglandins, thromboxanes and leukotriens, too.<sup>[33]</sup>

Fetal membrane production of these eicosanoids may stimulate uterine contractions in late preterm delivery and eicosanoid activated type IV collagenase activity may play a role in PPROM.<sup>[34]</sup>

We also found that mean of energy intake in the second trimester of women with PPROM was more than women without PPROM ( $P < 0.001$ ).

Fat provides energy, indeed it is the most energy dense of all the macronutrients, with 1 g providing 37 kJ (9 kcal).<sup>[33,35]</sup>

Considering higher intake of SFA and PUFAs among women who experienced PPROM, the relationship between higher intake of energy and PPROM is reasonable. Specifically, the association between energy intake and risk for PPROM in the current study was independent of BMI, estimates of physical activity, and weight gaining (in the second trimesters).

Moreover, researchers have shown that energy-restricted diet increases plasma antioxidant capacity and is able to decrease lipid per oxidation together with the benefits related to weight loss.<sup>[36]</sup>

Another result obtained in this study was higher iron and calcium intake in women who experienced PPROM ( $P = 0.045$  and  $P = 0.006$  respectively). Although energy production is an important function

**Table 2d: Maternal intake of antioxidants in the first, second, and third trimesters of pregnancy**

Nutrients (trimesters)	Mean±SD		P
	No PPROM (n=570)	PPROM (n=17)	
A (mg) (the first trimester)	861.24±1055.02	922.11±1012.58	0.81
A (mg) (the second trimester)**	780.95±1067.45	1707.11±2929.92	0.001**
A (mg) (the third trimester)	711.27±1144.74	494.49±231.03	0.55
Au (the first trimester)	6957.13±7385.83	7577.11±5868.23	0.73
Au (the second trimester)	5935.62±6514.83	9334.39±19783.89	0.05
Au (the third trimester)	5031.77±5302.25	4458.44±1833.51	0.73
C (the first trimester)**	147.90±99.84	206.23±156.47	0.020**
C (the second trimester)**	152.70±105.76	208.66±193.10	0.037**
C (the third trimester)	183.76±137.80	172.04±81.59	0.78
Beta-carotene (the first trimester)	494.39±683.78	508.46±525.75	0.97
Beta-carotene (the second trimester)**	371.80±569.39	783.31±525.75	0.007**
Beta-carotene (the third trimester)	292.43±394.46	200.68±124.98	0.43
Carotenoid (the first trimester)	494.39±683.78	508.46±525.75	0.93
Carotenoid (the second trimester)**	371.80±569.39	783.32±1975.79	0.010**
Carotenoid (the third trimester)	292.43±394.46	200.68±124.98	0.46
E (the first trimester)	1.42±2.71	1.75±4.23	0.62
E (the second trimester)	1.53±3.00	2.37±5.00	0.26
E (the third trimester)	1.71±3.44	1.55±2.22	0.88

SD: Standard deviation, PPROM: Preterm premature rupture of membranes

**Table 2e: Maternal intake of micronutrients in the first, second, and third trimesters of pregnancy**

Nutrients (trimesters)	Mean±SD		P
	No PPROM (n=570)	PPROM (n=17)	
Mn (the first trimester)	6.27±4.86	5.63±4.29	0.59
Mn (the second trimester)	6.95±4.82	7.07±5.16	0.92
Mn (the third trimester)	6.87±4.59	6.97±5.10	0.94
Mg (the first trimester)	213.28±109.47	227.24±93.00	0.60
Mg (the second trimester)	231.21±121.98	275.09±91.11	0.14
Mg (the third trimester)	243.97±147.86	199.84±42.59	0.34
Cu (the first trimester)	3.02±5.77	2.97±5.44	0.97
Cu (the second trimester)	3.39±6.30	3.10±3.57	0.85
Cu (the third trimester)	2.63±4.70	1.37±1.37	0.39
Ca (the first trimester)	835.82±409.74	953.21±322.17	0.24
Ca (the second trimester)**	943.26±425.01	1234.20±426.14	0.006**
Ca (the third trimester)	921.91±422.74	854.57±216.65	0.61
K (the first trimester)	2929.87±1275.72	3177.81±1178.46	0.42
K (the second trimester)	3242.83±1321.58	3755.79±1440.66	0.11
K (the third trimester)	3184.80±1623.47	2430.33±692.16	0.14
Na (the first trimester)	2819.32±1542.97	3046.82±1739.75	0.55
Na (the second trimester)**	3080.73±1621.73	4252.58±2845.20	0.004**
Na (the third trimester)	3125.14±1644.01	2634.96±606.23	0.34
Fe (the first trimester)	18.68±8.58	21.10±9.24	0.11
Fe (the second trimester)**	20.47±8.79	24.87±11.59	0.045**
Fe (the third trimester)	20.56±10.40	20.74±9.01	0.95
Phos (the first trimester)	937.41±421.23	919.91±324.43	0.86
Phos (the second trimester)	1046.08±451.18	1129.41±318.65	0.45
Phos (the third trimester)	1100.26±814.60	967.42±338.84	0.60
Zn (the first trimester)	7.46±4.62	7.57±4.20	0.91
Zn (the second trimester)	0.36±6.54	9.50±4.49	0.47
Zn (the third trimester)	9.10±15.21	7.01±2.52	0.66
Se (the first trimester)	43.90±34.16	35.17±16.15	0.29
Se (the second trimester)	47.23±34.17	47.56±27.97	0.96
Se (the third trimester)	48.44±37.38	50.04±21.64	0.89

SD: Standard deviation, PPROM: Preterm premature rupture of membranes

**Table 3: The association of significant nutrients intake with PPROM by logistic regression**

Significant nutrients intake	Beta	Wald	P	OR
Ca (the first trimester)	0.003	1.782	0.002	1.02
Na (the second trimester)	0.0003	1.650	0.004	1.002
Fe (the second trimester)	0.034	0.774	0.007	1.041
En (the second trimester)	0.001	0.689	0.01	1.002
Beta-carotene (the second trimester)	0.001	0.597	0.018	1.001
Ca (the second trimester)	-0.001	0.521	0.03	0.999
Carotenoid (the second trimester)	-0.007	0.517	0.04	0.994
PUFA (the second trimester)	0.014	0.441	0.047	1.015
A (mg) (the second trimester)	0.0004	0.340	0.12	1.0001
SFA (the second trimester)	-0.014	0.190	0.19	0.989
C (the second trimester)	0.0003	0.008	0.22	1.001

OR: Odds ratio, SFA: Saturated fatty acid, PUFA: Poly unsaturated fatty acid, PPROM: Preterm premature rupture of membranes

of mitochondria, these organelles also participate in initiating and executing both apoptotic and necrotic cell death as well as in maintaining calcium and iron

homeostasis.<sup>[37]</sup> Thus, it appears that mitochondria serve as a central barometer for assessing changes in cellular viability. Mitochondrial-related events such as increased concentrations of free Ca<sup>2+</sup> and bioavailable ferrous iron appear to be important contributors to the demise of the cell and OS, either collectively or individually.<sup>[38,39]</sup>

In another study, researchers demonstrated that the burden of ROS can be further amplified by the presence of free metals such as iron, copper and manganese that are released from metalloprotein complexes.<sup>[40]</sup> Also Brion *et al.* reported that ROS production is enhanced by the presence of free iron.<sup>[41]</sup> A growing body of literature indicates that PPROM may result from ROS-induced damage to amnion epithelium or collagen in the chorioamnion.<sup>[26]</sup>

As mentioned above, mitochondrial matrix ca2+ overload can lead to enhanced generation of reaction oxygen species (ROS).<sup>[42]</sup> In conformity with our results about calcium, Iams *et al.* showed that although agents such as calcium or antioxidants were able to reduce preeclampsia, a resulting decrease in preterm birth was not observed and nor did the rate if PPROM in some studies.<sup>[43]</sup>

We also found that mean of sodium intake in the second trimester of women with PPROM was more than women without PPROM (P = 0.004).

There are two sodium-dependent membrane transporters encoded by SLC23A1 and SLC23A2, which have key roles in human Vitamin C metabolism and which control dietary uptake, re-absorption and tissue distribution of Vitamin C.<sup>[44]</sup> On this background, Erichsen *et al.* provided new data to link Vitamin C transport mechanisms, albeit indirectly, to preterm birth.<sup>[45]</sup> The mechanisms for Vitamin C uptake, re-absorption and distribution are dependent on sodium. Therefore, the relationship between higher sodium intake and the risk of PPROM can be attributed to Vitamin C absorption and distribution. Increasing Vitamin C and PPROM is also seen in the current study. However, these findings need to investigate biologically and cellularly, that was not possible in this study.

In accordance to the data, higher intake of Vitamin C, Vitamin A (mg), carotenoids, and β-carotene intakes were significantly associated to the risk of PPROM (P < 0.05).

There are two types of antioxidant in the human body: Enzymatic antioxidants and nonenzymatic antioxidants. The latter include glutathione,

**Table 4: Physical activity during the first, second, and third trimesters and risk of PPROM**

Physical activity	No PPROM				PPROM				Total				P
	Minimum	Maximum	Mean±SD	n	Minimum	Maximum	Mean±SD	n	Minimum	Maximum	Mean±SD	n	
Stage - First trimester	0.46	100.48	17.99±10.99	567	7.07	37.25	19.01±9.62	17	0.46	100.48	18.02±10.95	584	0.706
Stage - Second trimester	1.78	110.75	16.43±10.53	561	7.32	47.06	19.93±11.11	17	1.78	110.75	16.5187±10.57222	575	0.178
Stage - Third trimester	00	100.50	15.31±10.82	511	0.80	34.50	18.87±11.63	11	0.00	100.50	15.38±10.84	522	0.282

SD: Standard deviation, PPROM: Preterm premature rupture of membranes

Vitamins A, C and E. In agreement with our findings, Mathews and Neil have been able to demonstrate no protective effect of increased levels of antioxidant nutrients against PPROM.<sup>[46]</sup> The possibility of a pro-oxidative effect of  $\beta$ -carotene, Vitamins C, E and lipid acid as contributory factors to the observed negative effects have been also suggested.<sup>[47-49]</sup>

At the following, we will explain the above mentioned association of antioxidants and the risk of PPROM in detail.

An important finding of the present study are the significant differences in mean values if Vitamin C intake in patients with PPROM in the first and second trimester compared to other mothers ( $P = 0.02$ ,  $P = 0.03$ , respectively). In fact, Vitamin C intake in mothers with PPROM was significantly more than healthy mothers.

The elevated risk of preterm premature rupture of the membranes has been shown for women with a low Vitamin C intake in some studies.<sup>[50]</sup>

A Cochrane review on the effects of Vitamin C supplementation in pregnancy concluded that there was too little data to determine whether Vitamin C supplementation is beneficial and that it may be associated with PPROM.<sup>[50]</sup>

Also other studies reported an increased frequency of PPROM among women randomized to vitamin supplementation.<sup>[17,51]</sup>

Kumar *et al.*, s results indicate that hydrogen peroxide (HP) induces apoptosis in amnion-derived WISH cells (WISH cells have long been utilized as a model for human amnion epithelial cells from which they were thought to have been derived, the stability of this cell line makes it useful for studies that primary culture of amnion cells will not tolerate). WISH cells have long been utilized as a model for human amnion epithelial cells from which they were thought to have been derived, the stability of this cell line makes it useful for studies that primary

culture of amnion cells will not tolerate. Vitamin C preincubation does not inhibit, and may accelerate and exacerbate, HP induced apoptosis. It is imperative that detailed characterization of time and dose effects of Vitamin C and Vitamin E upon human chorio-amnion be completed before such agents are recommended routinely as a means to prevent preterm labor.<sup>[52]</sup>

Therefore, the association between higher intake of Vitamin C and PPROM may be explained by dose dependent pro-oxidant effects.

The last result showed the significant difference between the two groups in Vitamin A (mg), carotenoids, and  $\beta$ -carotene intake in the second trimester ( $P = 0.001$ ,  $P = 0.01$ , and  $P = 0.007$  respectively).

Vitamin A is considered to have antioxidant properties.<sup>[53]</sup> While not necessarily a causal relationship, some researchers have also observed an increased risk of PPROM among women with high serum levels of lutein.<sup>[46]</sup>

It is now becoming clear that there is an optimal dose of carotenoids that results in maximum antioxidant effectiveness in human cells, but it is still not evident why higher doses of carotenoid should be less effective than lower doses, or actually result in increased damage to the cell.<sup>[54,55]</sup>

Also, authors using cells in culture have shown not only loss of antioxidant effectiveness, but also pro-oxidant effects of carotenoids at high carotenoid concentrations.<sup>[56]</sup> Of the carotenoids,  $\beta$ -carotene has the highest provitamin A activity. The efficiency of  $\beta$ -carotene conversion to Vitamin A decrease, with higher  $\beta$ -carotene intake.<sup>[57]</sup>

Burton and Ingold more over than 30 years ago proposed that  $\beta$ -carotene may act as a pro-oxidant under conditions of high carotenoid and oxygen concentrations.<sup>[57]</sup> Also recently Deoliveira *et al.* concluded that vitamins such as  $\beta$ -carotene can exert an antioxidant effect and a pro-oxidant effect according to their concentrations, and could be an indicator of

an inflammatory process *in vitro* generating severe complications to the body in cellular levels.<sup>[58]</sup>

The study has a large sample size and prospective design. The work is unique in having collected data on a wide range of nutrients intake, and in measuring nutrients intake at three stages in pregnancy, which allowed investigating the possible changes in nutrients during gestation. Particular care was taken to assess potential confounding variables accurately, especially physical activity, demographic and reproductive characteristics.

We did not measure serum levels of nutrients, because it was not economically beneficial. Therefore, we recommend to assess the relationship between serum levels of macro- and micro-nutrients (demonstrated in the present study) and the risk of PPROM.

## CONCLUSION

Based on the results of this study, the mean value of received SFAs, poly unsaturated fatty acids, energy, iron, calcium, sodium, Vitamin C, A,  $\beta$ -carotene, Carotenoids in the second trimester and Vitamin C intake in the first trimester in subjects who experienced PPROM later in pregnancy was higher than the other pregnant women. Therefore, these findings could be considered in the nutritional programming for pregnant women to manage the risk of PPROM.

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## Conflicts of interest

There are no conflicts of interest.

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