Original Article

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Higher β-human chorionic gonadotropin and estrogen levels during the first 6 weeks of pregnancy are associated with threatened abortion

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Summary **The associations of human chorionic gonadotropin (hCG), estrogen, and progesterone levels with threatened abortion have not been fully studied. Eighty women with threatened abortion were recruited sequentially, and the levels in their pregnancy hormones during the first trimester were compared with that of 160 normal early pregnancy controls. The natural logarithm transformed (Ln) hCG and Lnestrogen of women with threatened abortion and gestational age ≤ 6 weeks were significantly higher than values for the normal controls of the same gestational age (8.6 ± 1.2 *vs*. 7.4 ± 1.7 mIU/mL and 5.8 ± 0.4 *vs*. 5.4 ± 0.5 pg/mL); the two hormones reached similar levels in the groups of gestational age > 6 weeks. Among the group with gestational age ≤ 6 weeks, a univariate logistic regression showed that LnhCG and Lnestrogen were associated with threatened abortion, with odds ratios (ORs) of 1.85 [95% confidence interval (CI): 1.30-2.64] and 4.62 (95% CI: 1.67-12.80), respectively. The multivariate logistic regression model revealed that hCG and estrogen were mutually confounding factors, and only hCG was an independent factor for threatened abortion (OR 1.56; 95% CI: 1.06-2.28). None of the variables in the univariate or multivariate logistic regression was a factor associated with threatened abortion after 6 weeks gestational age. In conclusion, β-hCG and estrogen levels in the first half of the first trimester are factors associated with threatened abortion.**

Keywords: Early pregnancy, cross-sectional study, gestational week

1. Introduction

The complex interaction among luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone regulates the follicular, ovulatory, and luteal phases of the menstrual cycle spatiotemporally (*1-3*). Levels of estrogen and progesterone are low at the beginning of the follicular phase, and then estrogen peaks and progesterone starts to increase during the ovulatory phase (1-3). Estrogen and progesterone levels are high during most of the luteal phase, which causes the lining of the uterus to thicken to prepare for possible fertilization (*1-3*). If no egg is fertilized, the corpus luteum degenerates and no longer produces progesterone, the estrogen level decreases, and a new menstrual cycle starts (*1-3*). This repertoire lasts throughout a woman's reproductive life, except during pregnancy (*1-4*).

A new repertoire occurs once a sperm meets an egg. Human chorionic gonadotropin (hCG) is synthesized, mainly by the syncytiotrophoblast of the newly developing placenta, 1 day after implantation. hCG replaces the function of LH on about day 8 after ovulation and rescues the corpus luteum from involution (*5*). This maintains progesterone and estrogen secretion by the ovarian granulosa cells during the first trimester (*6*). hCG is at its peak (about 100,000 IU/L) between weeks 8 and 10 of gestation, and tends to plateau at a lower level for the remainder

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of the pregnancy (*4,7*). Progesterone and estrogen are largely produced by the corpus luteum until about 10 weeks of gestation; after the first trimester, the placenta synthesizes and secretes these two hormones (*8-10*). Progesterone levels gradually increase to 100-200 ng/mL when the pregnancy reaches term gestation. Estrogen levels increase steadily during pregnancy and reach their peak in the third trimester (*8-10*).

Progesterone and estrogen play significant roles during a normal pregnancy (*8-10*). The gradually increasing level of estrogen during pregnancy enhances the formation of blood vessels and transfer of nutrients, and supports the developing fetus (*8-10*). Progesterone keeps the placenta functioning properly and the uterine lining healthy and thick, as well as stimulating growth of breast tissue (*4,7,8-10*). It also prevents natural pre-pregnancy contractions of the uterine smooth muscle, allowing the fetus to grow in the expanding womb. Progesterone stimulates secretion of Th2 and reduces secretion of Th1 cytokines, reducing maternal immunological rejection of the fetus (*11,12*).

Any change in the homeostasis of these hormones impacts a normal pregnancy (*13-15*). A low hCG level is always observed in women who eventually miscarried in their first trimester and can indicate a blighted ovum or an ectopic pregnancy, whereas a high hCG level can indicate a molar pregnancy or a multiple pregnancy (*13-15*). Threatened abortion often occurs during the period of luteal-placental shift (8 to 12 weeks of gestation) due to a limited corpus luteum function or an abnormality in placental progesterone production and secretion (*14*). A reduction in the rate of spontaneous miscarriage with the use of dydrogesterone was also observed (14). Lower estrogen level was found in women with threatened abortion and historically estrogen was also commonly used to save threatened pregnancies in these women (*16,17*). However, evidence from randomised controlled trials to assess the use of estrogen and/or progesterone for preventing miscarriages is insufficient (*6,13,17,18*). Thus, the associations of these hormones levels with abnormal pregnancy remain undefined, some theories are controversial (*6,13,17,18*), and further studies are needed. In this report, the associations of hCG, estrogen, and progesterone levels with threatened abortion were evaluated in 80 women with threatened abortion and 160 normal controls. Our results showed that β-hCG and estrogen levels in the first half of the first trimester are higher in women with threatened abortion than women with normal early pregnancy and no significant difference in progesterone level observed between two groups.

2. Materials and Methods

2.1. *Design and participants*

This study was carried out in accordance with the recommendations of the Declaration of Helsinki for medical research involving human subjects, the World Medical Association with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by The Review Board of the Ethics Committee of Medical Research at Minhang Hospital, Fudan University.

A cross-sectional study was conducted on continuously registered outpatients visiting our Department of Obstetrics and Gynecology, between January 2016 and February 2017. Integrating the current consensus on diagnosis of threatened abortion (*19-21*), in this report, threatened abortion (threatened miscarriage) referred to a bloody vaginal discharge or bleeding other than spotting during the first trimester of completed gestation with evidence of a progressive viable pregnancy on ultrasound.

For differential diagnosis of threatened abortion; a pelvic exam was performed on women suspected of threatened abortion to identify the source of bleeding and to determine whether the amniotic sac was ruptured; a transvaginal ultrasound was performed to determine the amount of bleeding, to learn intrauterine pregnancy, and to monitor the heartbeat and development of the fetus; serial quantitative serum β-hCG and progesterone levels were measured to monitor the dynamic changes of pregnancy hormones; in addition, gonorrhea and chlamydia testing were also performed to exclude infection. Since there is no reliable approach to diagnose threatened abortion, women suspected of threatened abortion, whose intrauterine fetus survived over first trimester, were identified as threatened abortion. All subjects were assembled by four experienced obstetricians with cross validation; any uncertainties in inclusion criteria were resolved by discussion among chief obstetricians in our department.

As showed in Figure 1, subjects with early pregnancy loss (the loss of a pregnancy during the first 13 weeks of pregnancy, $N = 9$), ectopic pregnancy ($N = 8$), twin or multiple pregnancy ($N = 2$), prophylactic hormonal supplementation ($N = 2$), and supportive hormonal care ($N = 3$) were excluded from this study. Exclusion criteria also include women with inevitable, incomplete, or complete abortion ($N =$); women with septic abortion ($N = 0$); and women with non-threatened abortion-related bleeding (such as extragenital bleeding, $N = 3$). Finally, 80 women with threatened abortion were included in this study.

The normal controls of early pregnancy were selected from every tenth women with an early pregnancy but no complications continuously (Figure 1). 14 subjects refuse to participate; 10 subjects with incomplete data; 3 subjects were ectopic pregnancy; and 3 subjects were twin/multiple were excluded from the control group. Finally, 160 subjects with early pregnancy were involved as normal controls.

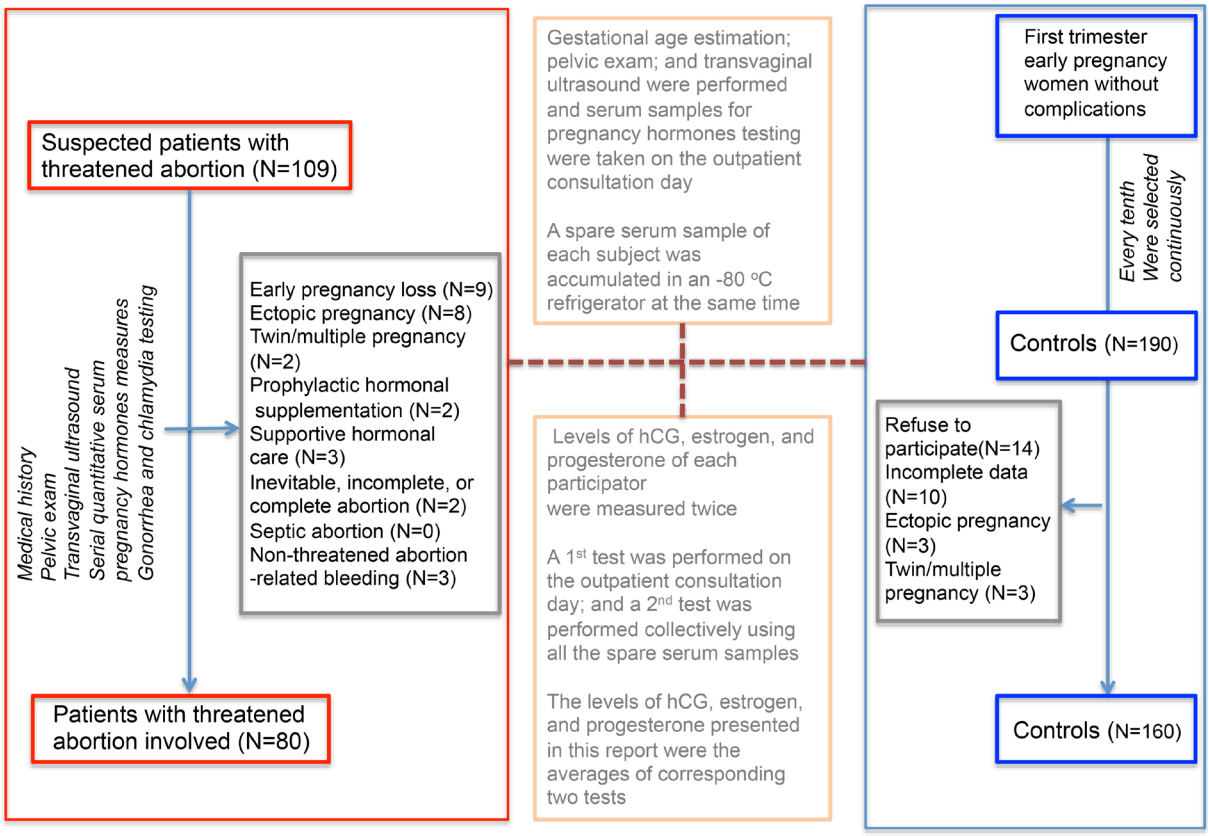

Figure 1. A diagram of the study subjects involved, measurements of pregnancy hormones and gestational age estimation. This is a cross-sectional study conducted on continuously registered outpatients visiting our Department of Obstetrics and Gynecology. Right panel, flowchart of controls involved; left panel, flowchart of cases involved; middle panel, diagnosis process.

Their levels of serum hCG, progesterone, and estrogen were measured according to the principle of ethical voluntariness. Gestational age was calculated from the last normal menstrual period if the mother had a regular period and knew the first day of her last menstrual period or according to the "Determination of Gestational Age by Ultrasound" recommendation from the American College of Obstetricians and Gynecologists (*19*) on the day visiting our department.

2.2. *Levels of serum hCG, progesterone, and estrogen*

The levels of hCG, progesterone, and estrogen were determined in the clinical laboratory of our hospital. Briefly, blood samples were drawn in all the women upon admission and centrifuged at 3,000 rpm at 4ºC in time; serum samples were stored at -20ºC before daily centralized and unified routine testing. For this study, a spare serum sample of each patient with strict records was accumulated in an -80 degree refrigerator at the same time. Levels of hCG, estrogen, and progesterone of each participator were measured twice; a 1st test was performed on the outpatient consultation day; and a 2nd test was performed collectively using all the spare serum samples; the levels presented in this report were the averages of corresponding twice tests (Figure 1). All measurements were carried out in the same laboratory. hCG, progesterone, and estrogen concentrations were measured using the ARCHITECT Total Beta hCG Reagent Kit (Longford, Ireland), the ARCHITECT PROGEST RGT, and the ARCHITECT Estrogen Reagent Kit according to the manufacturer's instructions. The inter-assay coefficients of variation were calculated from the mean values for the high and low controls (reference reagents provided by Abbott Architect) on each plate; the inter-assay coefficients of variation for estrogen, progesterone and hCG were 9.2%, 10.3% and 11.1%, respectively. The intra-assay coefficients variation were calculated from the individual coefficients variation for all of the duplicates; and the intra-assay coefficients variation for estrogen, progesterone and hCG were 7.9%, 6.8% and 8.3%, respectively.

2.3. *Statistical analysis*

The distribution of all variables is assessed using a histogram; variables with a skewed distribution were transformed by natural logarithm (Ln) and further presented by histogram. The Ln-transformed variables were used instead of the original clinical values. Ln-transformed and normally distributed variables are presented as means ± standard deviations (SDs). Differences between groups were evaluated using the independent-samples Mann-Whitney *U*-test. Spearman's

rank correlation coefficient array analysis was performed to determine the collinearity and interior relationships among the variables. Binary logistic regression analysis was adopted to understand how the pregnancy hormones were associated with threatened abortion. Variables in the models were selected according to Spearman's rank correlation coefficient and physiological and biochemical principles. All statistical analyses were performed with SPSS software (ver. 13.0; SPSS, Inc., Chicago, IL, USA), and the significance level was set to $\alpha = 0.05$.

3. Results

3.1. *hCG and estrogen levels are significantly higher in women with a threatened abortion*

Variables of pooled subjects (without stratification by gestational age) were compared first to detect differences in the pregnancy hormone levels between women with threatened abortion and those with normal early pregnancy. hCG, estrogen, and progesterone displayed skewed distributions, so the concentrations were natural log-transformed before statistical analyses. As shown in Table 1, the average Lnestrogen and LnhCG levels were significantly higher in women with threatened abortion than in those with normal pregnancy. No differences in age, gestational age, or progesterone levels were observed between the two groups. In conclusion, hCG and estrogen levels were significantly higher in women with threatened abortion.

3.2. *hCG and estrogen levels of women with a threatened abortion are higher in the first half of the first trimester*

The levels of hCG, estrogen, and progesterone were stratified by gestational age to further understand their distributions in women with threatened abortion and women with normal pregnancy. As shown in Figure 2A, the LnhCG level of women with threatened abortion and gestational age ≤ 6 weeks was 8.6 ± 1.2 mIU/mL, which was significantly higher than that of the normal controls (7.4 ± 1.7 mIU/mL) from the same gestational age group. Interestingly, the hCG level of women with threatened abortion was similar to that of normally pregnant women with gestational age > 6 weeks (10.8 ± 1.2 *vs*. 10.7 ± 1.2 mIU/mL). A similar trend in estrogen levels was observed: the Lnestrogen of women with threatened abortion and the normal controls of gestational age ≤ 6 weeks and > 6 weeks were 5.8 ± 0.4 *vs*. 5.4 ± 0.5 pg/mL and 6.4 ± 0.5 *vs*. 6.4 ± 0.6 pg/mL, respectively. The estrogen level of women with threatened abortion was significantly higher than that of the normal controls in the gestational age ≤ 6 weeks group (Figure 2B). The Lnprogesterone level of women with threatened abortion was similar between the two gestational age stratifications (2.9 ± 0.3 and 2.9 ± 0.3 ng/mL, respectively), whereas, the Lnprogesterone level of the normal controls tended to differ between the two gestational age stratifications (2.8 ± 0.5 and 3.0 ± 0.3 ng/mL, respectively; $P > 0.05$) (Figure 2C). Taken together, these data showed that the hCG and estrogen levels of women with threatened abortion were higher during the first half of the first trimester and reached levels similar to those of controls thereafter.

3.3. *Factors associated with threatened abortion without stratification by gestational age*

A multivariate logistic regression analysis was performed without the gestational age stratification to understand how hCG and estrogen levels were associated with threatened abortion. To exclude any impact of collinearity of these hormone indices on the statistical model, the relationships between individual variables were quantified by the Spearman's rank correlation coefficient array analysis. As shown in Figure 2D, hCG and gestational age, estrogen and gestational age, hCG and estrogen, and estrogen and progesterone were significantly correlated, with correlation coefficients of 0.871, 0.762, 0.787, and 0.255, respectively. Similar correlation coefficients were observed in women with threatened abortion, except for estrogen and progesterone (Figure 2E). Univariate analyses showed that LnhCG and Lnestrogen were possible factors associated with threatened abortion (Table 2). However, the multivariate logistic regression

Table 1. Differences between women with a threatened abortion and normal early pregnancy with no stratification by gestational age

Variables	Threatened abortion, $N = 80$	Early pregnancy, $N = 160$	P
Age	29.4 ± 5.3	28.9 ± 5.5	0.443
Gestational week (range)	6.7 ± 1.4 (4.6 - 11.0)	6.6 ± 1.6 (4.4 - 12.1)	0.217
hCG, mIU/mL	29,503.0 (10,825.0, 80,360.0)	12,026.6 (1,404.5, 52,255.8)	/
LnhCG, mIU/mL	10.0 ± 1.6	9.0 ± 2.2	< 0.001
Progesterone, ng/mL	19.2 (15.7, 21.0)	18.4 (14.9, 22.9)	/
LnProgesterone, ng/mL	2.9 ± 0.3	2.9 ± 0.4	0.570
Estrogen, pg/mL	480.0 (329.5, 844.0)	375.0 (220.0, 652.0)	/
LnEstrogen, pg/mL	6.2 ± 0.5	5.9 ± 0.7	0.001

Normally distributed data are presented as mean ± SD; skewed data are presented as median (interquartile range). Differences between groups were examined using an independent-samples Mann-Whitney *U*-test. hCG, human chorionic gonadotropin; Ln, natural logarithm.

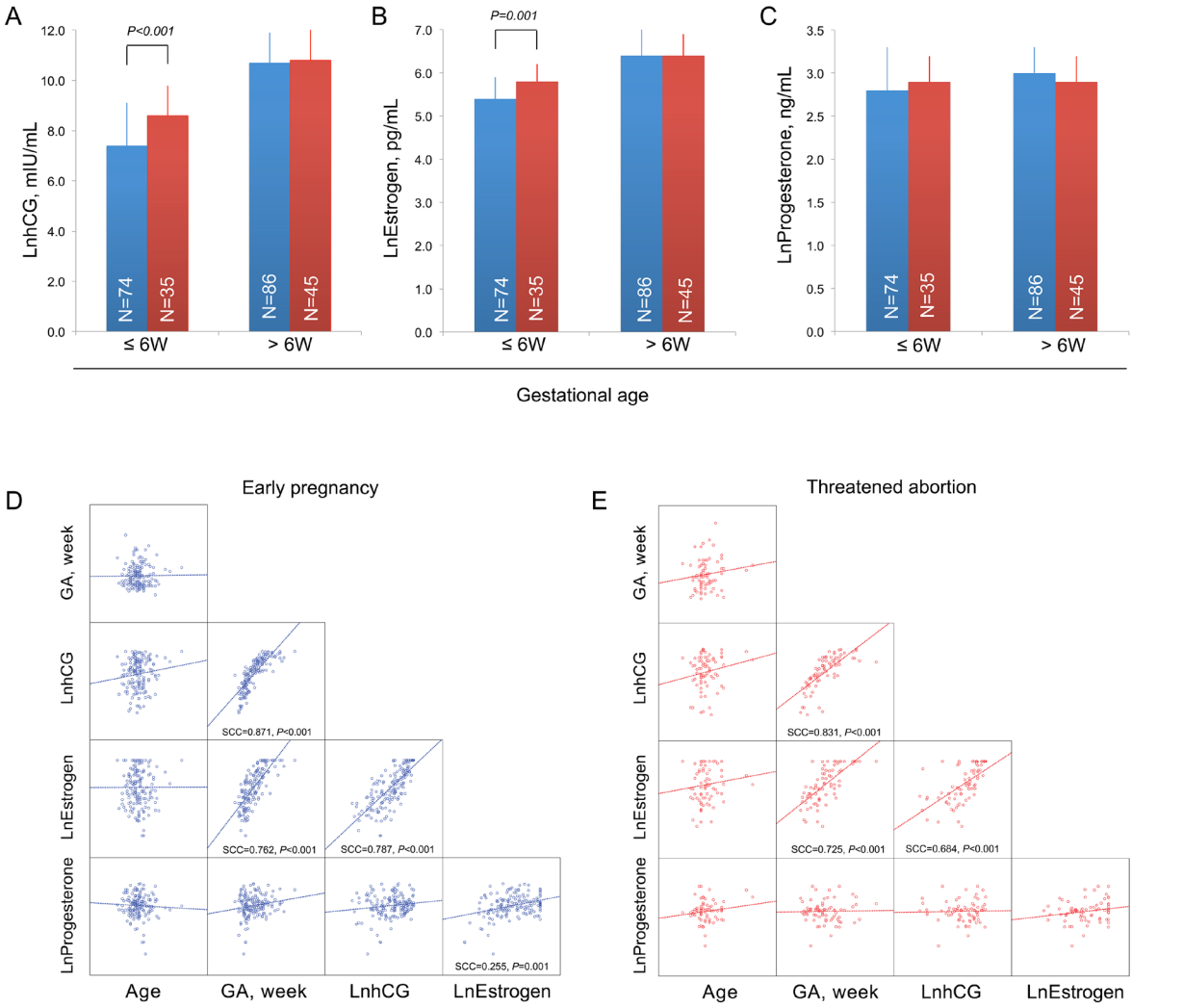

Figure 2. The levels in pregnancy hormones stratified by gestational age and Spearman's rank correlation coefficient array analysis. (A) to **(C)**, average LnhCG, Lnestrogen and Lnprogesterone of women with gestational age ≤ 6 weeks (numbers of threatened abortion and early pregnancy were 35 and 74, respectively) and > 6 weeks (numbers of threatened abortion and early pregnancy were 45 and 86, respectively). Red histograms in **(A)** to **(C)**, women with threatened abortion; blue histograms in **(A)** to **(C)**, women with normal early pregnancy. **(A)**, average LnhCG with SD; **(B)**, average Lnestrogen with SD; **(C)**, average Lnprogesterone with SD. **(D)**, collinearity and relationships between variables of women with early pregnancy were quantified by Spearman's rank correlation coefficient array analysis. **(E)**, collinearity and relationships between variables of women with threatened abortion were quantified by Spearman's rank correlation coefficient array analysis. SCC, Spearman's rank correlation coefficient; SD, standard deviation.

Table 2. Factors associated with threatened abortion without stratification by gestational age

Items	OR	95% CI	*P*
Univariate analyses			
Gestational week	1.34	0.97 - 1.55	0.213
LnProgesterone, ng/mL	1.24	0.59 - 2.58	0.569
LnhCG, mIU/mL	1.31	1.13 - 1.52	< 0.001
LnEstrogen, pg/mL	2.06	1.32 - 3.21	0.001
Multivariate logistic regression modeling			
LnEstrogen, pg/mL	1.89	0.91 - 2.31	0.235
LnhCG, mIU/mL	1.28	1.10 - 1.49	0.001

Backward stepwise (Wald) multivariate logistic regression analysis was adopted. hCG, human chorionic gonadotropin; Ln, natural logarithm; OR, odds ratio; CI, confidence interval.

model showed that hCG and estrogen were mutually confounding factors, and only LnhCG remained an independent factor positively associated with threatened abortion (OR 1.28; 95% CI 1.10-1.49; Table 2).

3.4. *Factors associated with a threatened abortion with stratification by gestational age*

The preceding analysis showed that hCG and estrogen

Table 3. Factors associated with a threatened abortion using stratification by gestational age

Items	Gestational age ≤ 6 weeks, *N* = 109			Gestational age > 6 weeks, *N* = 131		
	OR	95% CI	*P*	OR	95% CI	*P*
Univariate analyses						
Gestational week	2.35	0.93 - 5.72	0.521	0.95	0.70 - 1.28	0.739
LnProgesterone, ng/mL	2.11	0.71 - 6.31	0.188	0.49	0.15 - 1.64	0.252
LnhCG, mIU/mL	1.85	1.30 - 2.64	0.001	1.07	0.79 - 1.44	0.656
LnEstrogen, pg/mL	4.62	1.67 - 12.80	0.003	1.27	0.64 - 2.55	0.491
Multivariate logistic regression modeling						
LnEstrogen, pg/mL	2.59	0.86 - 7.79	0.091	1.38	0.62 - 3.07	0.437
LnhCG, mIU/mL	1.56	1.06 - 2.28	0.023	0.94	0.65 - 1.34	0.718

hCG, human chorionic gonadotropin; Ln, natural logarithm; OR, odds ratio; CI, confidence interval.

levels of women with threatened abortion were higher in the first half, but not in the second half, of the first trimester. To study the factors associated with threatened abortion more precisely, subjects were divided into two groups: gestational age ≤ 6 weeks and > 6 weeks. A logistic regression analysis was performed on these two groups. The univariate analysis showed that LnhCG and Lnestrogen were factors for threatened abortion in the group with gestational age ≤ 6 weeks. The multivariate logistic regression model showed that hCG and estrogen were mutually confounding factors, and only LnhCG remained an independent factor positively associated with threatened abortion (OR 1.56; 95% CI 1.06-2.28; Table 3). As expected, none of the hormone levels was a factor associated with threatened abortion for the group of gestational age > 6 weeks in the univariate or multivariate logistic regression model (Table 3).

4. Discussion

Abnormal early pregnancy is common and has numerous clinicopathological characteristics (*21,22*). In this study, we focused on threatened abortion and evaluated the associations of three hormones with threatened abortion. The LnhCG and Lnestrogen levels of pooled subjects with threatened abortion were significantly higher than those of gestational age-matched controls with normal pregnancy. A further gestational age stratification showed that LnhCG and Lnestrogen levels were significantly higher than the normal controls only in women with threatened abortion at gestational age ≤ 6 weeks. Univariate analyses revealed that LnhCG and Lnestrogen were factors associated with threatened abortion. Although hCG and estrogen were mutually confounding factors and only LnhCG remained an independent risk for threatened abortion in the multivariate logistic regression model, we incline to results of univariable analyses to annotate the role of hCG and estrogen in association with threatened abortion based on following physiological principle: hCG is synthetized and secreted by the syncytiotrophoblast of the developing placenta, whereas, progesterone and estrogen is synthetized and secreted by corpus luteum (*4,7,8-10*); although hCG is a dominant hormone to maintain corpus luteum to secrete progesterone and estrogen during the first trimester, the role of estrogen in association with threatened abortion could not be erased by the high collinearity between hCG and estrogen levels.

As serum hCG levels normally double every 1.8-3 days for the first 6-7 weeks of pregnancy, any error in the gestational age calculation may have affected our comparative analysis (*23,24*). However, no significant difference in average gestational age was observed between the case and control groups. The univariate analysis also showed no association between gestational age and threatened abortion. Thus, the matched gestational age excluded any possible effects of gestational age on these hormone levels between the groups. Although hCG increases sharply during the first half of the first trimester, estrogen increases at a steady and slow rate (*8-10*), so a higher estrogen level in the threatened abortion group was not likely caused by an error in the gestational age calculation. The average Lnprogesterone level was stable between ≤ 6 weeks and > 6 weeks of gestational age, which also suggested that the higher estrogen level was not a secondary error associated with higher hCG. Thus, higher Lnestrogen and steady Lnprogesterone levels might reflect an inherent pathophysiological characteristic of the corpus luteum associated with threatened abortion.

Current knowledge on high hCG levels is very limited. Existing hypotheses posit an error in the gestational age calculation, a signal of a twin or multiple pregnancy, and a result of fertility drugs (*22*). Molar pregnancy or trisomy 21 may cause excessive beta-hCG (*21,25,26*); since all participants had undergone ultrasound examination to monitor the heartbeat and development of the fetus; a possibility that the presence of molar pregnancy in the threatened abortion group could be excluded. While, trisomy 21 screening has not been carried out in our hospital, so, we could not exclude a possibility that the presence of trisomy 21 in the threatened abortion group was responsible for higher hCG partially. Nonetheless, all of our subjects

were singleton pregnancies, and women taking fertility drugs were excluded from this study. We also excluded the possibility of error in gestational age calculation in the preceding paragraph. Thus, a higher level of hCG in the first 6 weeks likely represents an abnormal pathophysiological characteristic in the synthesis and secretion of hCG associated with threatened abortion.

The definition of miscarriage includes types of inevitable, complete, missed and recurrent miscarriages in addition to threatened abortion. As a type with relatively good prognosis, threatened abortion is not studied enough. Commonly used estrogen and/or progesterone supplements had not approved by randomised controlled trials (*6,13,17,18*) suggesting that our knowledge in this topic is insufficient. Our data showed that higher, not lower, hCG and/or estrogen levels associated with threatened abortion. Although a cross-sectional study could not reveal the pathological mechanism of these associations, these clinical evidences will enlighten studies on the mechanism.

As we know, LH, FSH, and hCG are heterodimers consisting of a common glycoprotein α-subunit and a unique β-subunit; and the β-subunits of LH and hCG shared 80% similarity in their amino acid sequences (*27*). During early pregnancy, hCG could control the function of corpus luteum through hypothalamic-pituitary-ovarian axis (*27*). The mechanisms underlying the elevation of hCG and estrogen in threatened abortion might be the following: higher hCG level might reflect the excessive development of placenta in women with threatened abortion, which altered the normal spatio-temporal interaction process between endometrium and placenta and cause bleeding; higher hCG level stimulates LH/hCG receptors on the granulosa-lutein cells (estrogen secreting), which are in a high steroidogenesis state of 18 carbon atoms steroids during the first 6 weeks of pregnancy (*27*), to produce and secret more estrogen; whereas the thecal-lutein cells (progesterone secreting) maintain a normal rhythm of progesterone synthesis and secretion; alternatively, the above pathological processes will trigger the maternal injury repair mechanisms, elevated estrogen level may be a manifestation of the maternal injury repair mechanisms (*28*). As hCG level peaked at 6-8 weeks and followed by a relatively sharp decline in the first trimester, the above physiological changes will thus be regressed in the 2nd half of first trimester; this might be why hCG and estrogen levels were significantly higher than the normal controls only in women with threatened abortion at gestational age ≤ 6 weeks, but not at gestational age > 6 weeks.

During the first trimester, most factors that cause a miscarriage are genetic (*21*). Although other reasons for miscarriage are varied and most often cannot be identified (*21*); the synthesis and secretion of hCG and luteal-placental shift are main endocrinological repertoire occurred in first trimester pregnancy, abnormality in the series of rigorous physiological processes are believed to be a causer of threatened abortion (*6,14,21*). This is why our study focused on the three hormones levels only in the first trimester pregnancy. Although all fetuses of women with threatened abortion survived over first trimester, some of them might end with spontaneous abortion after 13 weeks (*21,29*). Meanwhile, miscarriage after first trimester might also occur in our control subjects. Since the pathophysiology of miscarriage differed by trimesters (*19-22*), factors associated with miscarriage within remain two trimesters need to be evaluated in further study.

In conclusion, our data showed that higher β-hCG and estrogen levels during the first 6 weeks of gestation were associated with threatened abortion. These results will advance our understanding of hormone supportive care in women participating in assisted reproduction techniques.

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