

Relation of IL-6 expression in human placental tissue and fetal viability in Iraqi idiopathic preterm deliveryYasmin L. Alsaadi¹, Samia A. Eleiwe²¹ Faculty member- Department of Biology, College of Science, Baghdad University, Baghdad, Iraq.² Faculty member- Department of Anatomy, Histology & Embryology, College of Medicine, Al- Mustansiriyah University, Baghdad, Iraq.
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Abstract: Background: Preterm birth (PTB) is a worldwide public health dilemma that has significant undesirable effects on neonatal mortality and/or morbidity. In Iraq, idiopathic PTB prevalence seemed to be continued to increase and it stays put an imperative public health; hence any research in this context could deliver a new strategy in the prevention, detection and management of this important scene. **Aims:** This work was designed to compare the expression of interleukin-6 (IL-6) in placental tissue from Iraqi women who had either spontaneous term delivery; or idiopathic preterm delivery using immunohistochemistry (IHC) technique; in relation to fetal viability. **Methods:** Ninety placentas were collected from labor room at Department of Obstetrics and Gynecology of Al-Yarmook Teaching Hospital and Al-Khadhraa Private Hospital meant for vaginal delivery. These placentas were divided into control group consisted of 30 mothers had spontaneous alive term deliveries at completed 37th to 40th week of gestation and another two groups each consisted of 30 mothers had preterm deliveries (PTD), with length of pregnancy less than completed 36th week; one group of them were belonged to PTD with alive neonates and the other one were belonged to PTD gave stillbirths. All samples were fixed in 10% formalin solution and were managed as routine paraffin sections. Then used for immunohistochemical tests. **Results:** The mean immunoreactivity score (IRS) values of IL-6 at the placental decidual cells and blood vessels were significantly higher ($P < 0.05$), nevertheless its value in the villous trophoblasts was very highly significant ($P < 0.0005$) in PTD with stillbirths as compared to each of PTD having alive neonates and term alive deliveries (control group). **Conclusion:** Immunoreactivity score values of IL-6 expression in the human placental tissue were significantly increased in PTD with stillbirths, suggesting that IL-6 could be a useful marker for early detection and diagnosis of fetal bad outcomes and PTD. The importance of this cytokine to pregnancy and labor process may be illustrated by their activities in acceleration of labor process.

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Keywords: interleukin-6 (IL-6), Preterm birth (PTB), immunohistochemistry (IHC), preterm delivery (PTD)

1. Introduction

The placenta serves as the basic metabolic bringer to the fetus including nourishment, respiration, and excretion by means of being as a temporary liver, lung, and kidney. It is the organ for the interchange of substance between maternal and fetal blood streams without mixing or corporeal contact of the two blood streams [1]. The World Health Organization defines PTB as birth before 37 completed weeks of gestation. There are geographical differences in the incidence of PTB from country to country [2]. The concern of human labor remains a matter of debate till now[3]. Nevertheless, the role of inflammatory processes seems to be definite and leukocytes, possibly attracted by an increasing cell adhesion molecule expression, so, invade the myometrium, placenta, cervix, and fetal membranes at or immediately after the onset of labor [4&5]. Also, high level of pro-inflammatory cytokines, including IL-6 would be present within the reproductive tissues and in spite of so many researches

in the cytokine-producing cells at reproductive tissues during pregnancy and parturition, especially through immune-histochemical technique (IHC), this issue is still a matter of interest[6-8]. Placenta synthesizes a diversity of cytokines, to offer an additional level of complexity to the immune metabolic network of the pregnant mother particularly at the third trimester of pregnancy [9]. The expression of IL-6 is set up in both trophoblastic and endothelial cells [10&11]. IL-6 and its receptors expressed at the feto-maternal interface whether it was within the decidua or trophoblastic cells; are known to play a significant role in regulating a variety of placental functions[12]. IL-6 is a protein that acts as an endocrine hormone, immune-regulatory peptide and growth factor [11]. As one of the Pro-inflammatory cytokines, the IL-6 inhibits activity of human placental 11 β -hydroxysteroid dehydrogenase which probably increases intrauterine cortisol level which may drive prostaglandins production. Together these turn on the uterus, trigger the release of uterine

contractile stimulants to induce delivery [13&14]. Also, cortisol stimulates the manufacture of corticotrophin releasing hormone (CRH) in the placenta which may affect each of fetal and maternal responses to stress. Increased CRH intensity during pregnancy is associated with preterm labor [15&16].

Aim of the study

The aim of this research is to assess the expression of pro-inflammatory cytokines namely IL-6 in human placental tissues seen with term and idiopathic PTD, in a sample of Iraqi women, by IHC technique; and to find any relation with the fetal viability.

2. Material and Methods

The present study was performed at Department of Pathology in association with Department of Obstetrics and Gynecology at Al-Yarmook Teaching Hospital and Al-Khadhraa Private Hospital under supervision of Department of Anatomy, Histology and Embryology, College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq from March 2015 to the February 2016 which included 90 Iraqi women aged 20-35 years. Of course a verbal consent was taken from every mother included at this work. Thirty of these women had preterm vaginal delivery provided alive neonates, other thirty had preterm vaginal delivery with stillbirth, given that WHO defines PTB as birth prior to 37th wk of gestation [2], and thirty were the control group including a term spontaneous vaginal delivery (less than 37th wk of gestation). Gestational age was determined by the obstetrician depending on the first day of the last menstrual period as well as ultrasound examination, and was validated by neonatologist assessment at birth [17].

All of mothers were selected as uncomplicated either term; or preterm put alive neonate, or else preterm having stillbirths. Placentas were collected directly after normal vaginal delivery and sampling of placental tissues were taken just from the intermediate fraction of the cotyledon half length between the maternal aspect namely decidua basalis and fetal aspect that is chorion, in order to avoid the structural variation in tissues from parabasal and subchorionic areas [18]. The center part of the cotyledon was recognized by the characteristic central subchorionic blood lake [19]. This portion was cut at an area just on mid-way between the attachment of the umbilical cord and the border on the largest diameter of the placenta [20]. The samples were fixed with 10% formalin for 24-48 hours and were managed as routine paraffin sections. An average thickness of 5µm of the sections was used for immunohistochemical tests. Scoring for all the immune expression results were assessed with the aid of specialist pathologists. Positivity was

evaluated semi- quantitatively by the intensity and percentage of staining. Score of IL-6 expression was determined according to a scale when the cytoplasm had a brown color. The percentage of positively stained cells (PP) of the villous trophoblast, decidua cells, and blood vessels was evaluated in each field by two observers as the followings: 0 (<10% stained cells), 1 (≥10%), 2 (≥25%), 3 (≥50%), and 4 (≥75%). Staining intensity (SI) was graded as: 0 = no expression, 1 = weak, 2 = moderate and 3 = strong. The immunohistochemical expression of IL-6 was evaluated by using the scoring method known as immuno-reactivity score (IRS). According to this method, the percentage number of positively (PP) stained cells was multiplied by number of staining intensity (SI) score of positively stained cells to provide a combined immuno-reactivity score value (IRS) so $IRS = PP \times SI$, (21,22 & 23). Statistical analysis of the obtained data was carried out by using (SPSS Version 20). The statistical significance of results was assessed using the Student's t-test and ANOVA.

3. Results:

Immunohistochemical expression of IL-6:

The positive immunohistochemical expression of IL-6 was perceived in 22 out of 30 (73.3%) cases of term delivery (control group), 20 out of 30 (66.7%) cases of PTB had living neonates, and 20 out of 30 (66.7%) cases of PTB had stillbirths. The pattern of staining was cytoplasmic (Figs. 1, 2&3).

IL-6 expression in relation to villous trophoblast:

Interleukin-6 expression in term delivery was noticed in 7 out of 22 (31.8 %) cases within score 1, 10 out of 22 (45.5 %) cases within score 2 and 5 out of 22 (22.7%) cases within score 3. Regarding intensity of IL-6; it was demonstrated that 10 out of 22 (45.5%) cases within grade 1 and 12 out of 22 (54.5%) cases within grade 2.

In PTB with alive neonates; IL-6 expression was noticed in 3 out of 20 (15.0%) cases within score 1, 6 out of 20 (30.0%) cases within score 2 and 11 out of 20 (55.0%) cases within score 3. The intensity of IL-6 was 9 out of 20 (45.0%) cases within grade 1 and 11 out of 20 (55.0%) cases within grade 2.

In PTB with stillbirths IL-6 expression was noticed in 1 out of 20 (5.0%) cases within score 1, 7 out of 20 (35.0%) cases within score 2, 9 out of 20 (45.0%) cases within score 3, and 3 out of 20 (15.0%) cases within score 4. The intensity of IL-6 was 2 out of 20 (10.0%) cases within grade 1, 6 out of 20 (30%) cases within grade 2, and 12 out of 20 (60.0%) cases within grade 3, as shown in table (1).

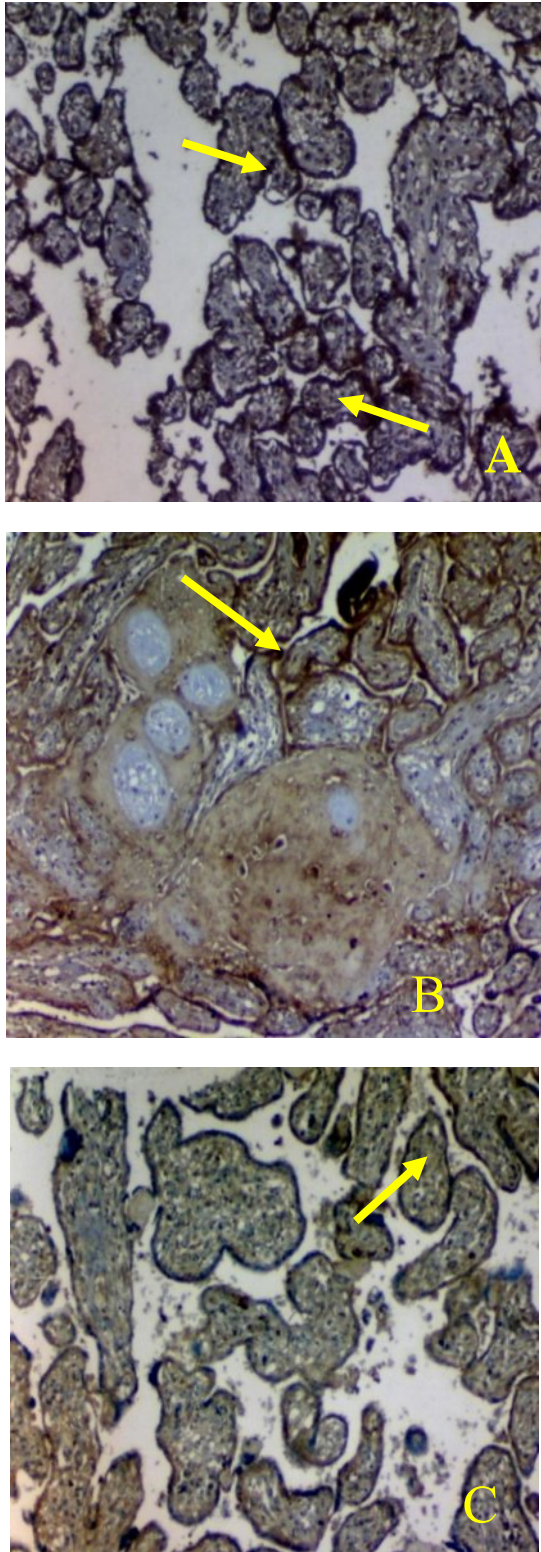


Figure 1: Immunohistochemical cytoplasmic positive expression of IL-6 in villous trophoblast: A: Strong cytoplasmic expression of IL-6 (X100), B: Moderate cytoplasmic expression of IL-6 (X 100), C: Weak cytoplasmic expression of IL-6 (X100)

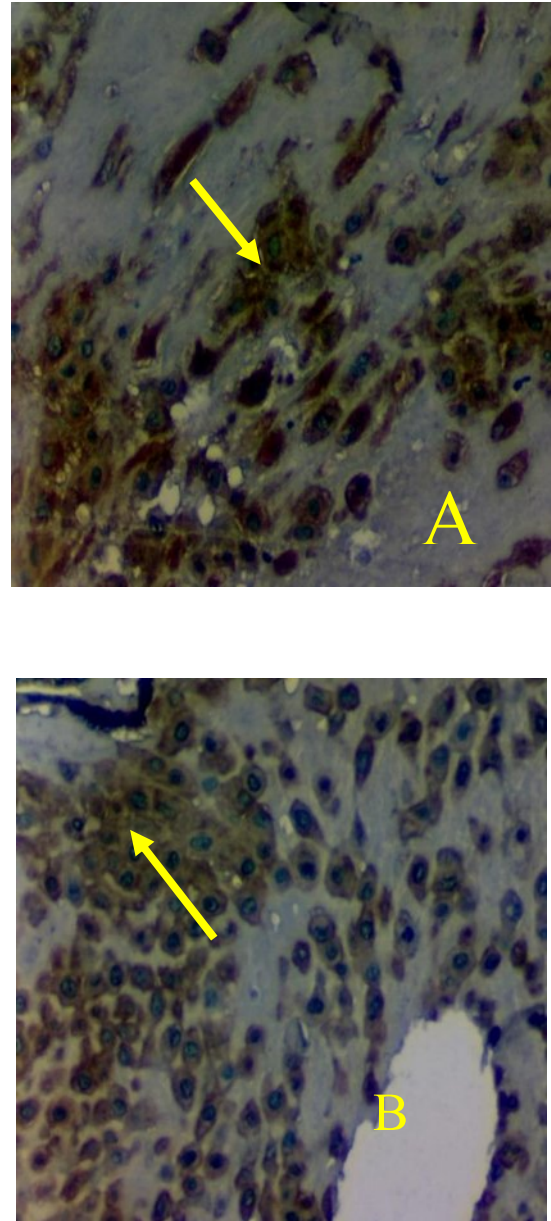


Figure 2: Immunohistochemical cytoplasmic positive expression of IL-6 in decidual cells
A: Moderate cytoplasmic expression of IL-6 (X400),
B: Weak cytoplasmic expression of IL-6 (X 400)

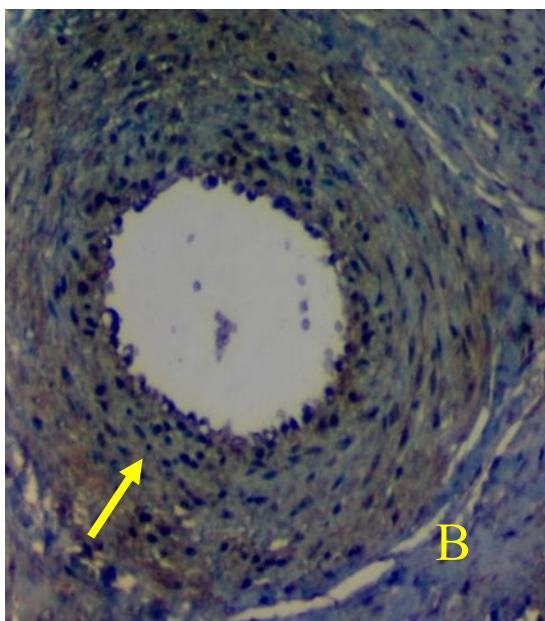
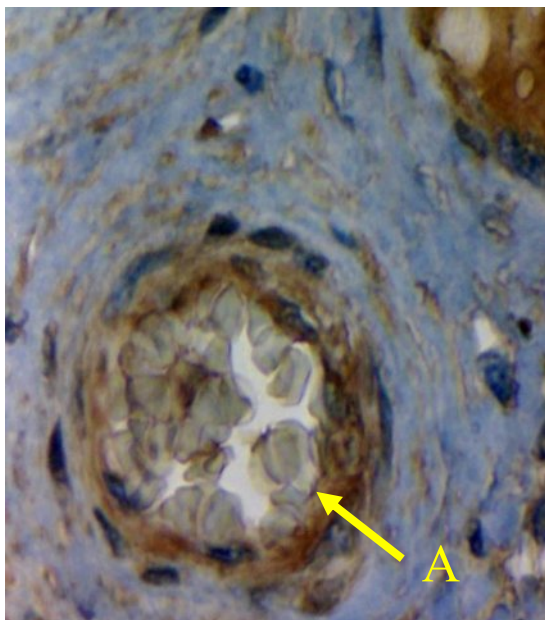


Figure 3: Immunohistochemical cytoplasmic positive expression of IL-6 in blood vessels:

A: moderate cytoplasmic expression of IL-6 (X400),
B: weak cytoplasmic expression of IL-6 (X100)

IL-6 expression in relation to decidual cells:

IL-6 expression in term delivery was 7 out of 22 (31.8 %) cases within score 1, 10 out of 22 (45.5%) cases within score 2 and 5 out of 22 (22.7 %) cases within score 3. The IL-6 intensity was 10 out of 22 (45.5%) cases within grade 1 and 12 out of 22 (54.5%) cases within grade 2.

In PTD with alive neonates IL-6 expression was 2 out of 20 (10.0%) cases within score 1, 9 out of 20 (45.0%) cases within score 2 and 9 out of 20 (45.0%) cases within score 3. The IL-6 intensity was 12 out of 20 (60.0%) cases within grade 1 and 8 out of 20 (40.0%) cases within grade 2. In PTD with stillbirths; the IL-6 expression was 12 out of 20 (60.0%) cases within score 2, 6 out of 20 (30.0%) cases within score 3, and 2 out of 20 (10.0%) cases within score 4. The intensity of IL-6 was 5 out of 20 (25.0%) cases within grade 1 and 15 out of 20 (75.0%) cases within grade 2 (Table 2).

IL-6 expression in relation to blood vessels:

IL-6 expression in term delivery was noticed in 3 out of 22 (13.6 %) cases within score 1, 12 out of 22 (54.5%) cases within score 2 and 7 out of 22 (31.8 %) cases within score 3. The intensity of IL-6 was 11 out of 22 (50.0%) cases within grade 1 and 11 out of 22 (50.0%) cases within grade 2. In PTD got alive neonates; IL-6 expression was noticed in 5 out of 20 (25.0%) cases within score 1, 3 out of 20 (15.0%) cases within score 2, 9 out of 20 (45.0%) cases within score 3 and 3 out of 20 (15%) cases within score 4. The IL-6 intensity was 9 out of 20 (45.0%) cases within grade 1, 11 out of 20 (55.0%) cases within grade 2. In PTD with stillbirths; IL-6 expression was noticed in 3 out of 20 (15.0%) cases within score 1, 6 out of 20 (30.0%) cases within score 2, 8 out of 20 (40.0%) cases within score 3 and 3 out of 20 (15%) cases within score 4. Whereas intensity of IL-6 was 2 out of 20 (10.0%) cases within grade 1 and 18 out of 20 (90.0%) cases within grade 2 (Table 3).

Scoring of IL-6 in placental tissue of term and preterm:

The total IHC score for IL-6 was evaluated by using the scoring method of immunoreactivity score (IRS) [21]. According to that method, the mean IRS values of IL-6 in blood vessels and that in the decidual cells of preterm with stillbirths were significantly higher ($P < 0.05$) as compared to preterm having alive neonates and term placenta, while the mean IRS values of IL-6 in the villous trophoblasts of preterm having stillbirths were very highly significant ($P < 0.0005$) as compared to preterm having alive neonate and term placenta (Table 4).

Table 1. Immunohistochemical expression and staining intensity of positively stained trophoblastic villous cells by IL-6

Expression (%) of IL-6	Studied groups		
	Term delivery (Control) N=22	Preterm delivery (alive neonate) N=20	Preterm delivery (stillbirth) N=20
1 (10-24%)	7	3	1
2 (25-49%)	10	6	7
3 (50-74%)	5	11	9
4 ($\geq 75\%$)	0	0	3
Intensity graded			
Weak(1)	10	9	2
Moderate (2)	12	11	6
Strong (3)	0	0	12

Table 2. Immunohistochemical expression and staining intensity of positively stained cells with IL-6 in decidual cells

Expression (%) of IL-6	Studied groups		
	Term delivery (Control) N=22	Preterm delivery (alive neonate) N=20	Preterm delivery (stillbirth) N=20
1 (10-24%)	7	2	0
2 (25-49%)	10	9	12
3 (50-74%)	5	9	6
4 ($\geq 75\%$)	0	0	2
Intensity graded			
Weak(1)	10	12	5
Moderate (2)	12	8	15
Strong (3)	0	0	0

Table 3: Immunohistochemical expression and staining intensity of positively stained cells of IL-6 in blood vessels

Expression (%) of IL-6	Studied groups		
	Term delivery (Control) N=22	Preterm delivery (alive neonate) N=20	Preterm delivery (stillbirth) N=20
1 (10-24%)	3	5	3
2 (25-49%)	12	3	6
3 (50-74%)	7	9	8
4 ($\geq 75\%$)	0	3	3
Intensity graded			
Weak(1)	11	9	2
Moderate (2)	11	11	18
Strong (3)	0	0	0

Table 4: Immuno-reactivity score values for IL-6 expression in placental tissues.

Studied groups	IL-6 of Decidual cells	IL-6 of blood vessels	IL-6 of Villous trophoblast
	Mean IRS value \pm SD (95% C.I)		
Term delivery (control) N= 23	2.909 \pm 1.444 (2.890-2.928)	3.273 \pm 1.486 (3.253-3.293)	3.682 \pm 1.460 (3.662-3.701)
Preterm delivery (alive neonate) N=27	3.350 \pm 1.725 (3.325-3.374)	3.800 \pm 1.963 (3.772-3.827)	3.700 \pm 1.720 (3.676-3.724)
Preterm delivery (stillbirth) N=28	4.350 \pm 1.663 (4.327-4.373)	4.750 \pm 1.803 (4.725-4.775)	7.100 \pm 3.401 (7.052-7.148)
ANOVA F value (Pvalue)	4.353 (0.017)*	3.788 (0.028) *	14.48 (0.0001)**
*ANOVA (one way) significant at level of significance 0.05. ** very highly significant at level of significance 0.0005.			

4. Discussion

The ongoing findings could be because that the PTD is known to be accompanied by an increased manufacture of IL-6 by placental cells in the villi compared to that of spontaneous term deliveries [24] and It was stated that IL-6 level was significantly increased to about 3-6 times in chorionic, decidual and amniotic tissues from mothers with preterm deliveries than those from mothers with term deliveries [10&25]. Nevertheless, it was set that in the absence of chorioamnionitis, PTB could be associated with physically powerful immuno-reactivity to IL-6 at endothelial cells of the trophoblastic villi (26,27).

It was reported that as an inflammatory event; human birth is linked with creation of the pro-inflammatory cytokines such as IL-6 by the decidua and fetal membranes with an increased level in preterm birth[28] but others stated that quite a few pro-inflammatory cytokines, including IL-6 have been implicated in the start of spontaneous PTB[29].

Elevation of expression of IL-6 within the decidua and placenta is frequently evident with altered cytokine profiles of PTD[30]. The IL-6 is well known to be associated with labor and the decidua is regarded as a major resource of this cytokine and a strong expression of IL-6 in the decidual cells of idiopathic spontaneous preterm labor was present independent to the existence of infection [31]. It was affirmed that placental cells from uninfected PTD produce significantly larger amount of cytokines as IL-6 with the absence of intrauterine infection and PTB is always associated with elevated placental cytokine [32]. Also, it was demonstrate that the adoptive transmit of regulatory T-cells can hold back the inflammatory reaction in the placenta of induced PTB in the mice, which is accomplished by mediating the expression of IL-6 at the placental tissues[33]. although a previous study was stated that the maternal aspect of term placentas produces significantly elevated amounts of IL-6 matched up with the fetal side of term placentas in comparison with preterm placentas[34].

The IL-6 production is usually regulated at decidual tissue in normally termed pregnancy, and the IL-6 deficient results in delayed ordinary timing of delivery by about 24 hours, hence; IL-6 might be regarded as a key that hasten the events of birth [35]. However; placental IL-6 receptors were recognized in villous syncytiotrophoblast without significant differences between term and PTB[36], The IL-6 receptors were expressed by fetal endothelial cells of the placental villi and allied with term spontaneous labor and also with preterm labor in the absence of intrauterine infection [37, 38]. Yet; the level of IL-6 was statistically significantly higher in normal term placentas than in PTD placentas [39]. Conversely;

placental homogenates after birth of different gestational ages were examined for IL-6 level and found no significant differences in placental IL-6 levels [40].

This study concluded that expression of IL-6 in the placenta were altered in the preterm delivery samples compared with the term delivery samples and we suggest that IL-6 may play a critical role in the pregnancy outcomes, resulting in the pathogenic course of preterm delivery with either living neonate or stillbirth.

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