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Neutrophils and monocyte toll like receptors 2 and 4 expression in preterm versus term delivery

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Abstract

Pregnancy results in an increase in immune cells, especially monocytes, which enhances the innate immune system. The increase of inflammatory cytokines in pregnant women's amniotic fluid, can cause uterine contraction, is linked to preterm labor. These inflammatory responses are controlled by Toll-like receptors (TLRs), which are largely expressed on neutrophils and monocytes. This study aimed to determine the role of neutrophils and monocyte subsets, as well as their expression of TLR-2 and TLR-4 in women with preterm and full-term delivery. The study involved a total of 74 women, comprising of 29 preterm labor, 25 full-term labor, and 20 non-pregnant women. The distribution of three monocyte subsets, namely (CD14++CD16-), (CD14+CD16+), and (CD14-/dim CD16++) was measured. Also, the expression of TLR2 and TLR4 in monocytes and neutrophils was analyzed using flow cytometry. Non-classical monocytes and intermediate monocytes were significantly higher in the preterm group than the control and full-term groups (p=0.041, p=0.043, and p=0.004, p= 0.049, respectively). Women in the preterm group showed significantly TLR2 expression on nonclassical monocytes compared to the control and full-term groups (p=0.002, and p=0.010, respectively). Also, preterm group expression of TLR4 was significantly higher in classical monocytes and nonclassical monocytes in comparison to the control group (p=0.019, and p≤0.0001, respectively). Besides, TLR4 expression was significantly up regulated in the preterm group compared to full-term in non-classical monocyte subset (p<0.0001). Moreover, the expression of TLR-4 in neutrophils from the preterm group was statistically higher than expression from the full-term labor and control groups (p<.0001 for both). Such findings highlight the important role of monocyte subsets and neutrophils in activating the innate immune system and initiating strong pro-inflammatory responses that induce preterm labor. Additionally, TLR4 and TLR2 expressions on non-classical monocytes may be used as a marker to assess the probability of preterm labor.

Keywords: Toll-like receptors (TLR), non-classical monocyte, Preterm labor

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Introduction

The incidence of preterm birth, defined as delivery before 37 weeks of gestation, has become a cause for concern due to its significant impact. It stands as the primary contributor to both perinatal morbidity and death in nations across the spectrum of development.[1] Babies born prematurely often face immediate and lasting negative effects, alongside a rise in healthcare expenses.[2]

Delivery, whether at full term or prematurely, involves a physiological response characterized by the presence of inflammatory substances. It has been suggested that preterm labor occurs due to excessive inflammation, overriding the natural signals of fetal organ readiness for birth. Around half of preterm births are linked to infections and inflammation within the amniotic sac. Evidence from tissue analysis and microbial studies suggests that localized infection and inflammation could be key factors in causing preterm birth.[3]

Elevated levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), IL-6, and IL-8 have been observed in the amniotic fluid of pregnant women undergoing preterm labor, leading to the degradation of amniotic membrane integrity, uterine contractions, and cervical ripening.[4]

Pregnancy results in an increase in immune cells, especially monocytes. The International Union of Immunological Societies' Nomenclature Committee divided human monocytes into three categories, which are determined by the expression of CD14 and CD16. These subsets are classical ($CD14^{+}CD16^{-}$), intermediate ($CD14^{high}CD16^{+}$), and non-classical ($CD14^{low}CD16^{+}$) cells.[5, 6]

Classical monocytes have specialized functions in phagocytosis, reactive oxygen species (ROS) production, and release of inflammatory cytokines a reaction to ligand binding, such as lipopolysaccharide (LPS) to Toll-like receptors (TLRs).[7] Intermediate monocytes display activated cell properties, including enhanced expression of the major histocompatibility (MHC) class II antigens and intracytoplasmic TNF-α.[8]

In contrast, non-classical monocytes do not produce ROS and have limited phagocytic activity. However, upon activation by ligands such as LPS, viruses, or nucleic acids binding to TLRs, they release a lot of pro-inflammatory cytokines, including TNF-α and IL-1.[9]

Research studies suggested that TLRs, predominantly expressed on neutrophils and monocytes, regulate inflammatory responses when infection primes the immune system.[10, 11] TLR-4 has obtained attention, as it is playing a crucial role in host cell reactions to LPS from gram-negative bacteria. TLR-2, on the other hand, has broad specificity and can identify peptidoglycan, lipoteichoic-acid, lipoproteins and fungal-cell wall. When exposed to interferon (IFN) and granulocyte macrophage-colony stimulating factor (GM-CSF), both neutrophils and monocytes have been observed to exhibit increased gene expression of TLR-2 and TLR-4.[12]

During the initial stages of pregnancy, monocytes in circulation migrate to the decidua and differentiate into either macrophages or dendritic cells.[13] Consequently, the production of decidual immune effectors can affect the composition of peripheral blood monocytes. The majority of studies that have investigated levels of TLR expression throughout normal and difficult pregnancies have concentrated on the cells and tissues that make up the maternal-fetal interface, including the trophoblast, decidua and amniochorionic membranes.[14, 15]

The aim of this research was to explore whether variations in the distribution of monocyte subsets and neutrophils or the expression of TLR-2 and TLR4 could be indicative of preterm labor.

Patients and Methods

The study included a total of 74 women divided in three groups. Group 1 (the preterm group) involved 29 pregnant women who arrived in early labor and preterm delivery from 27 to less than 37 weeks “gestation". Group 2 full-term labor group (the term group) included 25 pregnant women who completed 37 weeks gestation and were in labor. Twenty normal non-pregnant women not complaining from any

medical condition made up the third control group.

Pregnant women who were preterm and had multiple pregnancies, fetal abnormalities, diabetes, hypertension, placenta previa, or intrauterine growth restriction were not included in the study. However, preterm and full-term pregnant women, had a single pregnancy, and did not have any chronic or gestational medical problems were eligible to be included in the study.

The study collected basic obstetric and clinical information from all study pregnant women, and calculated gestational age based on the first day of their last menstrual period. Additionally, a blood sample (5 ml) was taken from each participant and placed into tubes containing EDTA, processed within three hours of collection for complete blood count (CBC) and flow cytometric assessment.

Flow cytometric detection of monocytes subsets and their expression of TLR2 and TLR4

To detect monocyte subsets in blood samples, a process involved staining and incubation with specific monoclonal antibodies was performed. Specifically, a blood sample (100 µl) from each study participant was mixed with 10 µl of fluorescein isothiocyanate (FITC)-conjugated CD282 (TLR2), phycoerythrin (PE)-conjugated CD284 (TLR4), peridinin-chlorophyll-protein (Per-CP)-conjugated anti-CD16, and allophycocyanin (APC)-conjugated anti-CD14. All monoclonal antibodies were obtained from Becton Dickinson (BD) Biosciences, San Jose, USA, with the exception of CD284, which was obtained from eBiosciences, Thermo Fisher Scientific, San Diego, USA. The mixture was then incubated for 15 minutes at room temperature in the dark. Following this, red blood cells were lysed, and the cells were washed and resuspended in phosphate buffer saline (PBS). Finally, flow cytometric analysis was performed using an automated flow cytometry machine (BD FACS Calibur, BD Biosciences, USA) with Cell Quest software (BD Biosciences, USA). About 50,000 events were acquired. An isotype-matched negative control using anti-human IgG was employed for each sample. The granulocyte (neutrophils) and monocyte population were defined using forward and side scatter histogram. The expression of CD14 and CD16 was then evaluated within the monocyte population. As the next step, the classical monocytes ($CD14^{++}CD16^{-}$), intermediate monocytes ($CD14^{+}CD16^{+}$) and non-classical monocytes ($CD14^{-/dim}$ $CD16^{++}$) were gated. Following this, the study evaluated the expression of TLR2 and TLR4 on granulocytes, classical monocytes, intermediate monocytes, and non-classical monocytes. The expression was reported as a geometric mean of fluorescence intensity (MFI) as shown in Figure 1.

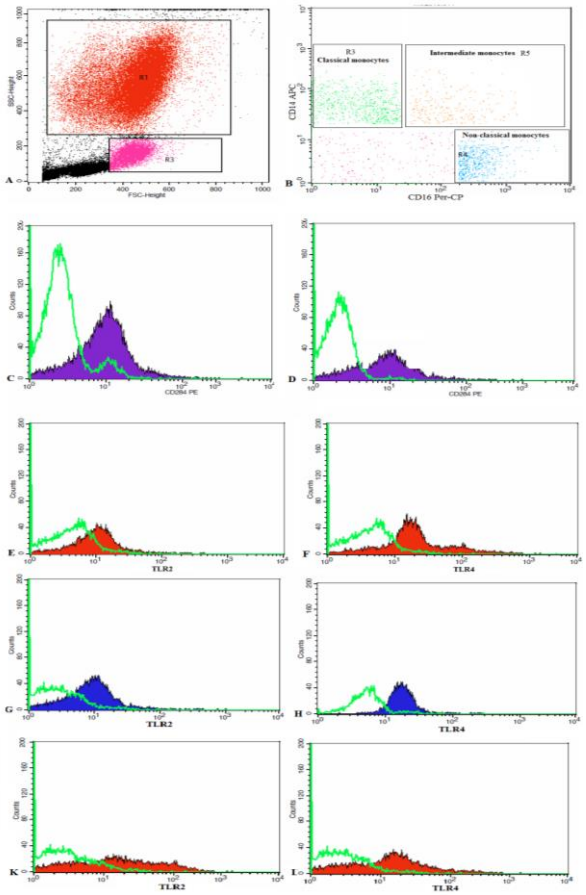

Figure 1. Detection of monocyte cells subtype by Flow cytometry. A: Forward and side scatter histogram was used to define the monocytes population (R3). B: cells were plotted for their CD14 and CD16 expression, after which three distinct subpopulations of monocytes were defined: $CD14^{++}CD16^{-}$ cells, $CD14^{+}CD16^{+}$ cells and $CD14^{-/dim}$ $CD16^{++}$ cells.

Statistical Analysis

The Statistical Package for Sciences software (SPSS), version 20.0, was used to conduct the statistical analyses. For continuous variables, the results are shown as means ± standard deviation (SD). Differences between two groups were examined by independent Sample T-test, while One Way ANOVA was employed to analyze continuous variables in more than two groups. A *p* value of less than 0.05 was considered statistically significant.

Results

Basic obstetric and clinical characteristics of the laboring women and the controls

The mean age did not significantly differ between the groups. Also, group1 (preterm labor) and group 2 (full-term labor) had significantly different gestational ages at the time of inclusion ($p< 0.0001$) as shown in Table 1.

Table 1. Characteristics of monocytes, monocyte subsets and neutrophils in laboring women in labor and the controls.

	Group 1 (preterm labor) (n=29)	Group 2 (full-term labor) (n=25)	Group 3 (non-pregnant control) (n=20)	*p* value		
				*p*1	*p* 2	*p* 3
Age (years)	28.20± 5.93	29.52±4.69	29.55±9.15	NS	NS	NS
Gestation age (weeks)	30.20± 3.17	37.84±.850				<0.0001
Monocytes (%)	11.11± 4.12	8.61± 2.44	7.01± 1.94	<0.0001	0.021	0.010
Classical monocytes (%)	81.37± 6.65	84.61± 4.56	85.09± 3.99	0.030	NS	0.045
Non classical Monocytes (%)	10.04±4.04	8.30±1.14	8.10±0.909	0.041	NS	0.043
Intermediate monocytes (%)	8.89± 3.99	7.17±1.64	6.04± 1.29	0.004	0.016	0.049
Neutrophils (%)	72.30± 12.10	60.92± 18.50	46.11±16.44	<0.0001	0.008	0.009

*p*1: group1 vs. group3; *p*2: group2 vs. group3; *p*3: group1 vs. group2. Data represented as means ± SD.
$p > 0.05$ is not significant (NS).

Analysis of the frequency of monocytes and monocyte subsets in study and control groups

Data in Table 1 show that the mean percentage of monocytes was significantly higher in preterm and term groups than in the non-pregnant control group. Also, a significant difference in monocyte percentage was observed between the preterm and the full-term group. The mean percentage of classical monocytes represented the largest mean within monocytes population. As the non-pregnant control group was the highest followed by term group with significant high difference between each group and preterm group. Non classical monocytes were significantly higher in the preterm group than the other two groups. The mean percentage of intermediate monocytes was significantly higher in the preterm and term groups than the non-pregnant control women. Also, the mean percentage of intermediate monocytes showed a significantly higher difference in preterm group than the term one.

Expression of TLR-2 and TLR-4 on the surface of different subsets of monocytes in study and control groups

We did not find any statistically significant difference in TLR2 expression neither on classical monocytes nor on intermediate monocytes among the three groups (p=0.104 and p=0.740, respectively). By contrast, a significant difference was detected in TLR2 expression on nonclassical monocytes among the three groups (p= 0.001). Consequently, this expression of TLR2 on nonclassical monocytes was significantly up regulated in the preterm group compared to term and control groups (p=0.010 and p=0.002, respectively). Additionally, TLR4 expression in each monocyte subset was significantly different among the three groups as shown in Figure 2. In the preterm group, the expression of TLR4 was significantly higher in classical monocytes and non-classical monocytes in comparison to the control group (p=0.019 and p< 0.0001, respectively). Besides, the expression of TLR4 in nonclassical monocyte subset was significantly up regulated in the preterm compared to the term group (p<0.0001). In contrast, intermediate monocytes, TLR4 expression in the term group was significantly higher in comparison to the control group (p=0.002).

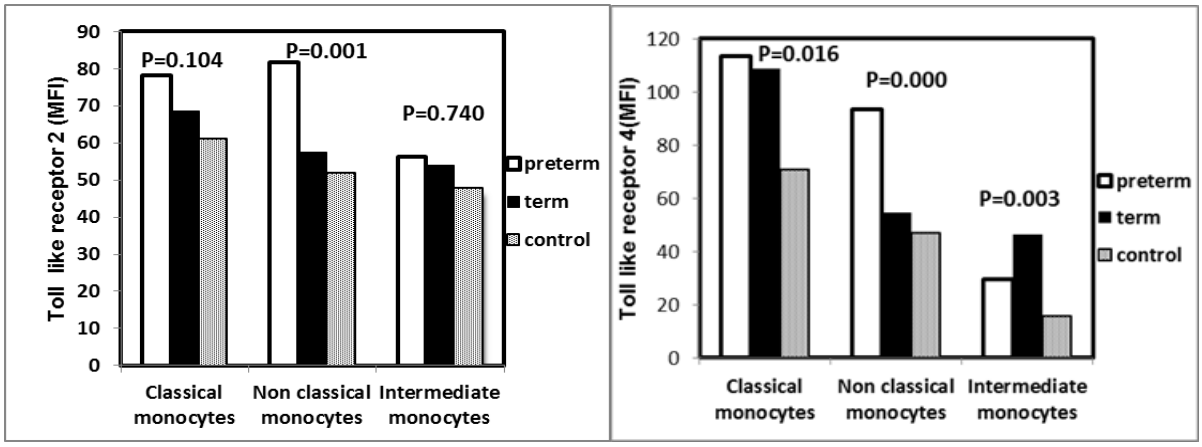

Figure 2. Expression of TLR-2 and TLR-4 on the surface of monocyte subsets in women in labor and controls.

Frequency and expression of TLR-2 and TLR-4 on the surface of neutrophils

Data in Table 1, show that in the preterm group the mean percentage of neutrophils was significantly higher than in the term and control groups. Moreover, the neutrophils mean percentage in term group was significantly higher than in the control group. The TLR4 expression in neutrophils was significantly different among the three groups (p<0.0001). The expression of TLR-4 in neutrophils from the preterm group was statistically higher than such expression from the term and control groups (p<0.0001 for both). We noted that the difference in the expression of TLR-2 in neutrophils was not statistically significant between any groups as shown in Figure 3.

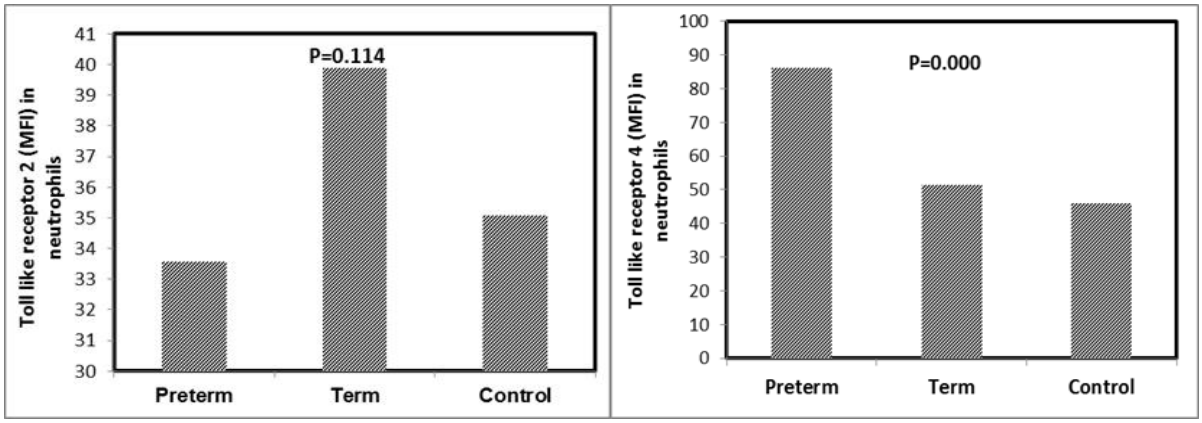

Figure 3. Expression of TLR-2 and TLR-4 on the surface of neutrophils in women in labor and controls.

Discussion

Despite progress in handling high-risk pregnancies, including those complicated by preterm birth, the occurrence of these issues remains elevated, and understanding the underlying mechanisms remains obscure. Our study was carried out to determine the role of neutrophils and monocyte subsets or their expression of TLR-2 and TLR4 in preterm labor.

The study revealed that the average percentage of monocytes was significantly higher in both preterm and term groups compared to the control group. As adaptive immunity is known to be diminished during pregnancy, the increased activation of innate immunity in the peripheral blood may be a necessary counterbalancing mechanism to ensure protection against infection.[16]

Our study found that classical monocytes were the predominant subset of monocytes, which is consistent with findings of the previous study by Kim et al., 2012.[17] In our study, we observed a significant decrease in classical monocytes in the preterm group compared to the non-pregnant control groups, which agreed with the findings of a study by Melgert et al., 2012 who reported that pregnant women had lower percentages of classical monocytes when compared to non-pregnant controls.[18] This may be due to the specialization of classical monocytes in phagocytosis, and the reduced phagocytic role of monocytes during pregnancy which can be a protective mechanism for the allogeneic fetus.[7, 19] However, the study by Al-Ofi et al., 2012 reported that pregnant women had more classical monocytes than non-pregnant women.[20]

Intrauterine infection has been closely linked with preterm birth.[21] Despite the thought that localized intrauterine infection is frequently asymptomatic, it has the potential to attract and activate monocytes as well as produce proinflammatory cytokines IL-1β and TNFα in response to bacterial products.[22] Our study found that the average percentage of intermediate monocytes was significantly higher in both preterm and term groups compared to the control group, with a higher difference observed in the preterm group. This is consistent with a previous study that reported a higher percentage of this subset in the preterm group compared to the full-term and pregnant control groups.[17] Additionally, evidence from other inflammatory diseases supports the existence of the intermediate subset (CD14 ^{high} CD16+), in large numbers.[8]

According to our research, the preterm group had a much larger percentage of non-classical monocytes than the other two groups. This finding is consistent with the analysis of an animal model, which showed a higher proportion of non-classical monocytes in pregnant rats than in non-pregnant rats.[18] However, another study reported a decreased number of non-classical monocytes in pregnant women compared to non-pregnant women.[20] A research study has shown that the non-classical monocyte subset has poor phagocytic function, despite producing significant amounts of pro-inflammatory cytokines such as TNF-α and IL-1β.[9]

A previous research study has demonstrated that intermediate/non-classical monocytes, which express CD16, are more pro-inflammatory than the classical subset.[23] The available data support the idea that preterm labor is a pro-inflammatory condition and that the activation of CD16+ monocytes in response to infectious stimuli may trigger the maternal pro-inflammatory response, ultimately leading to preterm labor. Furthermore, the high expression of the chemokine receptor CX3CR1 on CD16+ monocytes enable these cells to have a strong affinity for endothelial cells, facilitating their rapid extravasation in response to inflammatory stimuli.[24,25]

TLRs are primarily expressed on monocytes and are believed to regulate the maternal pro-inflammatory response,[26, 27] which is linked to early activation of the parturition pathway after TLR involvement.[28] Ilievski et al., 2007 demonstrated that TLR-2 ligands, such as lipoteichoic acid or peptidoglycan were sufficient to induce premature delivery in healthy pregnant mice.[29] Our research revealed that TLR-2 expression was significantly upregulated on non-classical monocytes in the preterm group compared to the term and control groups, as opposed to other monocyte subsets. This finding further supports the notion that these unique monocyte subsets may play a role in the initiation of labor.

This study found a significant difference in TLR4 expression among the three groups in each monocyte subset. Moreover, the preterm group had a higher percentage of TLR4 expression in both classical and non-classical monocyte subsets compared to the control group. These findings agreed with those of a study by Pawelczyk et al., 2010 which reported a significant increase in frequency of TLR4-positive monocytes and TLR4 mRNA expression in women in preterm labor.[27] Additionally, murine studies have demonstrated that LPS stimulates monocytes, leading to preterm labor via TLR4 interaction.[30] Conversely, blocking TLR4 to inhibit monocyte activation could significantly reduce LPS-induced preterm labor.[31]

Furthermore, in the present study, the preterm group showed significantly upregulated TLR4 expression on non-classical monocytes compared to the term group. The increased expression of TLR4 in monocytes of preterm women may signal the start of an inflammatory response in response to potential infectious stimuli, which could be associated with probable preterm labor. This is supported by findings of a study by Tang et al., 2015 which reported that TLR4 triggers genes that encode pro-inflammatory cytokines.[32]

In the present study, the expression of TLR-4 on neutrophils in the preterm group was significantly higher than in the term and control groups, while there was no statistically significant difference in the expression of TLR-2 among all groups. The study by Moço et al., 2018 found that the level of TLR-2 protein expression on neutrophils did not differ between preterm and non-laboring pregnant women. Furthermore, consistent with our results, TLR-4 protein expression was increased in maternal neutrophils during preterm labor.[10] The various bacterial species found in the amniotic cavity may be the cause of the difference in TLR-2 and TLR-4 expression by maternal neutrophils during preterm.[10]

In conclusion, our study findings together with the previously mentioned data, suggests that the non-classical monocyte subset together with TLR2 and TLR4 expression are specifically increased in preterm labor. This underscores the significant involvement of both monocytes and neutrophiles in triggering the innate immune system and instigating robust pro-inflammatory reactions leading to preterm labor. Moreover, the expression of TLR4 and TLR2 on non-classical monocytes could serve as an indicator for evaluating the likelihood of preterm labor.

Author Contributions

AMZ and ZAMZ performed the lab work, KMZ examined the patients and collected samples, ERB and AS writing and revision of the paper, HFH statistical analysis, YA paper revision. All authors participated in study design and paper reviewing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Faulty of Medicine, Assiut University, Egypt (approval dated March 2021).

Informed consent

A signed consent form was obtained from each study participant before being included in the study.

References

1. Simmons LE, Rubens CE, Darmstadt GL, et al. (2010). Preventing preterm birth and neonatal mortality: exploring epidemiology, causes, and interventions. *InSeminars in perinatology* 34(6): 408-415).

2. Petrou, S., Eddama, O. and Mangham, L. (2011). A structured review of recent literature on the economic consequences of preterm birth. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, 96(3):225-232.

3. Di Renzo GC, Tosto V, Giardina I. (2018). The biological basis and prevention of preterm birth. *Best Practice & Research Clinical Obstetrics & Gynaecology*.1(52):13-22

4. Menon, R., Taylor, R.N. and Fortunato, S.J., (2010). Chorioamnionitis–a complex pathophysiologic syndrome. *Placenta*. 31(2):113-120.

5. Passlick B, Flieger D, Ziegler-Heitbrock HW. (1989). Identification and characterization of a novel monocyte subpopulation in human peripheral blood. 2527–2534.

6. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood*. 116 (16):74-80.

7. Saha P, Geissmann F. (2011). Toward a functional characterization of blood monocytes. *Immunol. Cell Biol.* 89 (1):2-4.

8. Rossol M, Kraus S, Pierer M, et.al. (2012). The CD14brightCD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. *Arthritis Rheumatol.* 64(3):671-677.

9. Wong KL, Tai JJ, Wong WC, et al. (2011). Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood*. 118(5):16-31.

10. Moço NP, Batista RA, Martin LF, et al. (2018). Toll-like receptor-2 and-4 expression by maternal neutrophils in preterm labor. *Gynecol. Obstet. Invest*. 83(1):1-8.

11. Takeuchi O, Akira S. (2010). Pattern recognition receptors and inflammation. *Cell*. 140(6):805-820.

12. O'Mahony DS, Pham U, Iyer R, et.al. (2008). Differential constitutive and cytokine-modulated expression of human Toll-like receptors in primary neutrophils, monocytes, and macrophages. *Int. J. Med. Scip.* 5(1):1.

13. Svensson J, Jenmalm MC, Matussek A, et.al. (2011). Macrophages at the fetal–maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. *The JI*. 187(7):3671-3682.

14. Moço NP, Martin LF, Pereira AC, et al. (2013). Gene expression and protein localization of TLR-1,-2,-4 and-6 in amniochorion membranes of pregnancies complicated by histologic chorioamnionitis. Eur. J. Obstet. Gynecol. Reprod. Biol. 171(1):12-17.

15. Hoang M, Potter JA, Gysler SM, et al. (2014). Human fetal membranes generate distinct cytokine profiles in response to bacterial Toll-like receptor and Nod-likereceptor agonists. Biol Reprod; 90: 39.

16. Warning JC, McCracken SA, Morris JM. (2011). A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. *Reproduction*. 141(6):715-724.

17. Kim J, Ko Y, Kwon K, et al. (2012). Analysis of monocyte subsets and toll-like receptor 4 expression in peripheral blood monocytes of women in preterm labor. *J. Reprod. Immunol*. 94(2):190-195.

18. Melgert BN, Spaans F, Borghuis T, et al. (2012). Pregnancy and pre-eclampsia affect monocyte subsets in humans and rats. *PloS one*.7(9): e45229.

19. Lampé R, Kövér Á, Szűcs S, et al. (2015) Phagocytic index of neutrophil granulocytes and monocytes in healthy and preeclamptic pregnancy. *J. Reprod. Immunol.*107:26-30.

20. Al-ofi E, Coffelt SB, Anumba DO. (2012). Monocyte subpopulations from pre-eclamptic patients are abnormally skewed and exhibit exaggerated responses to toll-like receptor ligands. *PloS one*. 7(7):42217.

21. Romero R, Espinoza J, Chaiworapongsa T, et.al. (2002). Infection and prematurity and the role of preventive strategies. *Semin Fetal Neonatal Med*. 7(4): 259-274. WB Saunders.

22. Norman JE, Bollapragada S, Yuan M, et.al. (2007). Inflammatory pathways in the mechanism of parturition. *BMC pregnancy childbirth*. (1):1-4.

23. Mukherjee, R., Kanti Barman, P., Kumar Thatoi, P., (2015). Non-classical monocytes display inflammatory features: validation in sepsis and systemic lupus erythematous. *Scientific reports.* 5(1):13886.

24. Auffray C, Fogg D, Garfa M, et al. (2007). Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 317(5838):666-670.

25. Gautier EL, Jakubzick C, Randolph GJ. (2009). Regulation of the migration and survival of monocyte subsets by chemokine receptors and its relevance to atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. 29(10):1412-1418.

26. Kopp E, Medzhitov R. (2003). Recognition of microbial infection by Toll-like receptors. *Curr. Opin. Immunol.* 15(4):396-401.

27. Pawelczyk E, Nowicki BJ, Izban MG, et al. (2010). Spontaneous preterm labor is associated with an increase in the proinflammatory signal transducer TLR4 receptor on maternal blood monocytes. *BMC Pregnancy Childbirth*.10(1):1-9.

28. Agrawal V, Hirsch E. (2012). Intrauterine infection and preterm labor. *Semin Fetal Neonatal Med.* 17(1): 12-19. WB Saunders.

29. Ilievski V, Lu SJ, Hirsch E. (2007). Activation of toll-like receptors 2 or 3 and preterm delivery in the mouse. *Reprod Sci.* 14(4):315-320.

30. Lin Y, Xie M, Chen Y, et.al. (2006). Preterm delivery induced by LPS in syngeneically impregnated BALB/c and NOD/SCID mice. *J. Reprod. Immunol.*71(2):87-101.

31. Li L, Kang J, Lei W. (2010). Role of Toll-like receptor 4 in inflammation-induced preterm delivery. *Mol. Hum. Reprod*. 16(4):267-272.

32. Tang MX, Hu XH, Liu ZZ, et.al. (2015). What are the roles of macrophages and monocytes in human pregnancy? *J. Reprod. Immunol.* 112:73-80.