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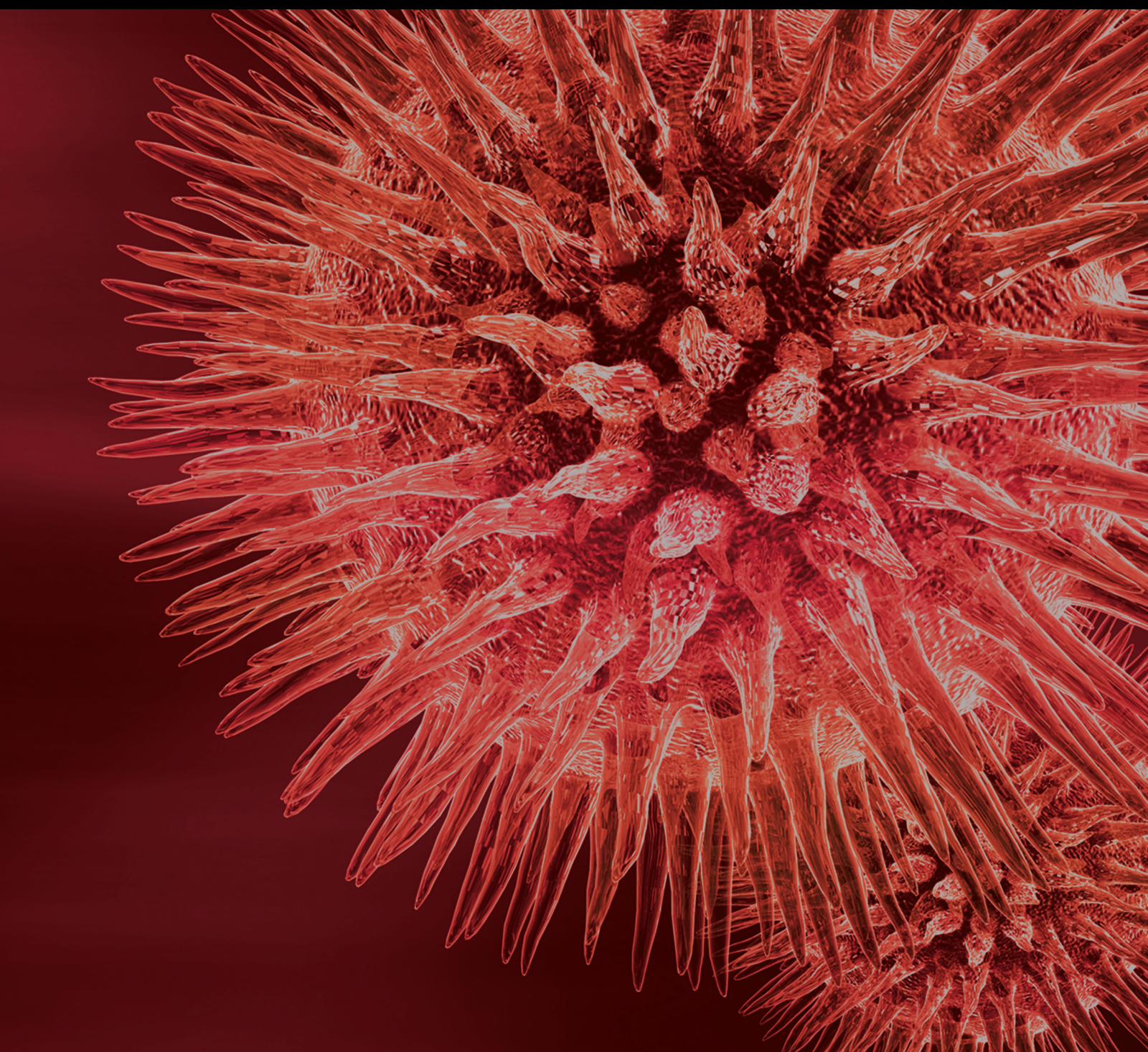
Preterm Labor: Up to Date

Lead Guest Editor: George J. Daskalakis
Guest Editors: Birgit Arabin, Aris Antsaklis, and Luis Cabero Roura



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Editorial

Preterm Labor: Up to Date

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Preterm birth is a syndrome with many causes [1]. The rates of preterm births (PTB) have increased to approximately 15 million cases per year [2]. Despite the fact that current efforts of primary, secondary, and tertiary interventions try to decrease the rates of PTB, the prevalence of prematurity still ranges between 5% in some high-income countries and more than 20% in some low-income countries such as in sub-Saharan Africa and South Asia.

Perinatal mortality, neonatal mortality, and neonatal and long-term morbidity are inversely associated with gestational age at birth. Frequent neonatal complications include periventricular leukomalacia, necrotizing enterocolitis, respiratory distress syndrome, jaundice and kernicterus, neonatal infections, and prolonged hospitalization. Since the quality of neonatal care additionally affects the outcome, there is a vicious circle of poor perinatal and neonatal healthcare with long-term consequences for the economy in all societies, but mainly within poor countries or within subgroups of low socioeconomic status of higher income countries. A history of preterm birth is the most dominant risk factor; however, most determinants from the maternal history such as smoking, nutritional depletion, short interpregnancy interval, or advanced maternal age are only weakly associated with PTB. Meanwhile, transvaginal sonography of the cervix is used as a screening tool to indicate precocious cervical ripening by using centile values of the cervical length.

Iatrogenic preterm birth as caused by fetal growth restriction and/or preeclampsia has to be differentiated from spontaneous onset of preterm birth. The purpose of the present special issue is to highlight specific aspects of PTB.

In a first review article, B. Staude et al. investigated the pathophysiologic background that correlates altered microbiome to neonatal complications, including retinopathy, necrotizing enterocolitis, disrupted psychomotor development, and autonomic regulation. The authors report that breast milk is of detrimental importance during early human development as the maternal microbes that are excreted from the mammary gland and absorbed by direct contact of the skin surrounding the areola support the neonate to establish a rich diverse microbiome. The authors conclude that this is an ongoing field of research. Respiratory distress syndrome (RDS) is a predominant complication of prematurity. Its prevalence remains high in preterm neonates, despite the institution of tocolytics, corticosteroids, and surfactant in current clinical practice. In the present article A. Niesluchowska-Hoxha et al. investigated the impact of several risk factors on the rate of RDS by multivariable analysis [3]. Their findings suggest that female gender decreased the odds of developing RDS by approximately 50%, whereas abnormal fetoplacental circulation and fetal distress detected by abnormal uterine artery and middle cerebral artery Doppler increased the risk for RDS.

Research in gestational diabetes (GDM) has proven the emerging role of adipokines in high risk pregnancies [4]. In their present article R. Mierzynski et al. investigated the efficacy of serum adiponectin and omentin-1 levels in

predicting PTB in patients with GDM. The authors reported that omentin-1 may be used to predict preterm birth as they estimated that an increase of omentin level by 100 ng/ml decreases the possibility of preterm birth by almost 75%. On the other hand, adiponectin did not seem to correlate with preterm birth. Further investigation is needed.

Inflammation is a significant determinant of PTB. The nuclear factor-κB (NF-κB) has been implicated in the pathogenesis of PTB as a mediator. Downstream regulatory element antagonist modulator (DREAM) is a regulator of the NF-κB in nongestational tissues. P. Goradia et al. investigated DREAM expression in primary myometrial and amnion cells and observed that DREAM mRNA expression is increased and that, in myometrial and amnion cells, DREAM regulates proinflammatory and prelabour mediators. The authors conclude that DREAM is a promising factor that may be used in the future in the diagnosis and management of preterm labour.

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have also been associated with an increased risk of PTB. N. Jancar et al. investigate this correlation in a large population sample that included 267 718 women from Slovenia. They observed that IVF-ICSI nearly tripled the risk of early PTB (OR 2.8) and doubled the risk of late PTB (OR 1.7). Given this information, the authors suggested that women undergoing IVF-ICSI should be closely followed during pregnancy to allow early identification and management of cases at risk.

Next to cervical length the detection of the uterocervical angle (UCA) by transvaginal sonography has been investigated to detect a risk for PTB. In our systematic review we sought to investigate the published evidence and identified 11 relevant articles. Thereby it was observed that reduced UCA during the second trimester of pregnancy seems to be an independent factor that may help predict PTB <34 weeks. Nevertheless, significant heterogeneity was noted in terms of the investigated outcomes and UCA cut-off values.

Several methods have been introduced as preventive strategies for PTB in women with a shortened cervix, including vaginal progesterone, 17OH progesterone caproate, cervical cerclage, and cervical pessary [2]. Among these methods, both, cervical cerclage and pessary placement, require expertise to ensure optimal outcomes. N. Vasudeva et al. reviewed 39 patients that underwent elective McDonald cerclage (26 cases) or an emergency cerclage (13 cases). The authors noted the significant impact of cerclage in reducing PTB rates and reported that there is a need for a surveillance program to optimize outcomes.

In conclusion, we hope that the present issue helps to provide some update in the field of PTB that may be used to guide current clinical practice and expand future research.

Conflicts of Interest

B. Arabin has a direct ownership interest in the company that designed and produces and now distributes the Arabin pessary. The company is privately held and the profit is used to support the Clara Angela Foundation. The remaining authors report no conflicts of interest.

George Daskalakis
Birgit Arabin
Aris Antsaklis
Luis Cabero Roura

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Research Article

Is IVF/ICSI an Independent Risk Factor for Spontaneous Preterm Birth in Singletons? A Population-Based Cohort Study

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Guest Editor: Birgit Arabin

The aim of our study was to explore the risk factors for very preterm (gestation under 32 weeks) and moderate preterm birth (gestation weeks 32-36 6/7) in singleton pregnancies in a national retrospective cohort study. We also wanted to establish whether IVF/ICSI is an independent risk factor for preterm birth after adjusting for already known confounders. We used data for 267 718 singleton births from 2002-2015 from the National Perinatal Information System of Slovenia, containing data on woman, pregnancy, birth, the postpartum period, and the neonate for each mother–infant pair. Mode of conception, maternal age, education, BMI, parity, smoking, history of cervical excision procedure, history of hysteroscopic resection of uterine septum, presence of other congenital uterine malformations, bleeding in pregnancy, preeclampsia or HELLP and maternal heart, and pulmonary or renal illness were included in the analyses. Unadjusted OR for very preterm birth after IVF-ICSI was 2.8 and for moderate preterm birth was 1.7. After adjusting for known confounders, the OR was still significantly elevated (1.6 and 1.3, respectively). Risk factors for very preterm birth with OR higher than 2.4 were history of cervical excision procedure, resection of uterine septum, operation or having other congenital uterine malformations, and bleeding in pregnancy. Risk factors for very preterm birth with OR between 1.4 and 2.1 were age >35 years, being underweight or obese, not having professional education, smoking, first birth, preeclampsia/HELLP, and IVF/ICSI. Risk factors for moderate preterm birth with OR higher than 2.4 were history of cold knife conization and other congenital uterine malformations. We found that even after adjustment, IVF/ICSI represents a single risk factor for early and late preterm birth even after adjustment with other risks such as maternal age, smoking, or a history of invasive procedures for either cervical intraepithelial neoplasia or infertility treatment.

1. Introduction

Assisted reproductive technology is now widely used for treatment of different female and male causes of infertility. More and more babies are born after IVF/ICSI procedures each year worldwide. The same is true for Slovenia, where up to 4% of babies are born after IVF yearly [1]. IVF was first found to be connected with preterm birth, predominately because of increased percentage of multiple pregnancies. With the preferred use of single embryo transfer, the percentage of multiple gestations was significantly reduced [2]. But even singleton pregnancies after IVF were found to be connected with preterm birth [3–9].

Multiple factors were found so far to be connected with preterm birth, the most prevalent being extremes of maternal age, low maternal BMI, maternal smoking, infections, history of cervical excision procedures, uterine anomalies, infertility treatment, and others [10, 11].

Women conceiving after IVF/ICSI are a special population of pregnant women. Due to many years of infertility, they are older than women conceiving spontaneously. The infertility itself is a known risk factor for preterm birth [12], since different disorders (endometriosis, adenomyosis, polycystic ovary syndrome, and uterine fibroids) and unexplained infertility share inflammatory pathways, hormonal aberrations, decidual senescence and vascular abnormalities

that may impair pregnancy success through common mechanisms [13]. These patients also have a history of several gynecologic operations before their pregnancy.

The aim of our study was to explore the risk factors for very preterm birth, before 32 weeks of gestation, and moderate preterm birth, from 32 to 36 6/7 weeks of gestation, in singleton pregnancies in a large 14-year national study. We wanted also to further explore weather pregnancy after IVF/ICSI is an independent risk factor for very preterm birth and moderate preterm birth after adjusting for already known important confounders.

2. Materials and Methods

We conducted a population-based retrospective cohort study using data from Medical Birth Registry, the National Perinatal Information System of Slovenia (NPIS). We followed the methods of Jančar et al. 2016 [14]. NPIS contains data on woman, pregnancy, birth, the postpartum period, and the neonate for each mother–infant/infants pair. Data is collected at the time of birth in all 14 maternal hospitals in Slovenia according to standardized methodology and premade definitions of over 100 different social, health, and perinatal variables [15]. The National Perinatal Information System of Slovenia includes all live deliveries regardless of child's gestation and birth eight. Besides, all stillborn with birthweight of at least 500 g or gestational age of at least 22 weeks or both are included in the system. Registration is mandatory by law since NPIS also serves as Slovenia's medical birth registry. Data is sent to the Slovenian National Institute of Public Health on a yearly basis, where it goes through statistical quality checks, is edited, and forms the basis for the official perinatal statistics of Slovenia. In the study period 99.9% of women were delivered in a hospital.

This retrospective cohort did not need ethical approval according to Slovenian law [16].

The study population consisted of Slovenian residents who gave birth to singletons from January 1, 2002, to December 31, 2015. In this period there were 282 517 births in Slovenia. After exclusion of foreigners, Slovenian residents had 281 358 births in this period. After exclusion of 9800 multiple gestations (3.5% of all births), 3 836 (1.4%) induced births and elective cesarean sections before 37 weeks of gestation due to maternal and fetal conditions, and 4 cases with gestational week at the time of birth not recorded, our final sample for the analysis consisted of 267 718 spontaneous births of singletons.

To be able to analyze factors associated with spontaneous preterm birth, we excluded all induced births and elective cesarean sections before 37 weeks of gestation, which were carried out due to maternal or fetal illness or condition, such as preeclampsia, maternal chronical illness, intrauterine growth restriction, or other critical conditions. Only births with a spontaneous onset have been included.

Outcome variables were spontaneous preterm birth before 32 weeks and spontaneous preterm birth between 32 and 36 6/7 weeks of gestation. Gestational age was determined according to the last menstrual cycle and first ultrasound in pregnancy by obstetrician or gynecologist, in agreement with the paediatrician assessment after birth. In cases of unclear gestational age, it was determined individually, considering the anamnesis of last menstrual period, including possible alterations such as irregular cycles, combined with the first available ultrasound estimation and findings of the pediatrician. The most probable gestational age was included in the system.

Along with mode of conception, IVF/ICSI or spontaneous, a total of 11 other covariates obtained from NPIS were included in the analyses: maternal age, maternal education, maternal BMI, parity, smoking during pregnancy, history of cervical excision procedure, history of hysteroscopic resection of uterine septum, presence of other congenital uterine malformations, bleeding in pregnancy, preeclampsia or HELLP in pregnancy and maternal heart, and pulmonary or renal illness. We selected those covariates, because they have been previously reported to affect the risk for preterm birth [10]. Maternal age was categorized into five groups: younger than 25 years, 25–29 years, 30–34 years, 35–39 years, and 40 years or older. Maternal education was categorized into five groups: primary or less, vocational, secondary or professional, tertiary, and not stated. Maternal BMI was categorized into four groups: less than 18.5 kg/m^2, 18.5–24.9 kg/m^2, 25–29.9 kg/m^2, and 30 kg/m^2 and higher. Parity was categorized into three groups: first birth, second birth, and third or more. Reported smoking during pregnancy was categorized into two groups: no or yes. History of cervical excision procedure was categorized into three groups: no, history of cold-knife conization, history of newer excision procedures, and predominately large loop excision of transformation zone (LLETZ). History of hysteroscopic resection of uterine septum was categorized into two groups: no or yes. Presence of other congenital uterine malformation was categorized into two groups: no, when there was no anomaly, and yes, when it was present or when it had been surgically corrected before this pregnancy. Bleeding in pregnancy was categorized into two groups: no and yes, when there had been a history of bleeding anytime in this pregnancy. Preeclampsia or HELLP in this pregnancy was categorized into two groups: no or yes. A history of maternal heart and pulmonary or renal illness was categorized into two groups: no or yes.

Chi-square test was used for descriptive analysis. Logistic regression analyses were performed to estimate crude odds ratio (OR) and adjusted odds ratio (aOR) and their 95% confidence intervals (95% CI) with two-sided probability (p) values. A p value of < 0.05 was considered statistically significant. For statistical calculations, we used IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp.).

3. Results

In the study period a total of 5 837 (2.2%) singletons were conceived after IVF/ICSI. The percentage of pregnancies after IVF/ICSI rose constantly over the years: from 1.5% in the year 2002 to 3.9% in the year 2015. The characteristics of our study population according to distribution of covariates divided by mode of conception is shown in Table 1.

Table 1: Characteristics of women included in the analysis, Slovenia, 2002–2015.

Characteristic	*Spontaneous conception n = 261 881*		*IVF/ICSI n = 5 837*		*p value*	*All births n = 267 718*	
Maternal age (years)	***n***	**%**	***N***	**%**	***<0.001***	***n***	**%**
< 25	40150	15.3	79	1.4		40 229	15.0
25 – 29	97348	37.2	1006	17.2		98 354	36.7
30 – 34	87586	33.4	2496	42.8		90 082	33.6
35 – 39	31625	12.1	1782	30.5		33 407	12.5
40 ≥	5172	2.0	474	8.1		5 646	2.1
Maternal education					***<0.001***		
Primary or less	11 174	4,3	197	3.4		11 371	4.2
Vocational	35 525	13,6	779	13.3		36 304	13.6
Secondary or professional	89 836	34,3	1 849	31.7		91 685	34.2
Tertiary	92 322	35,3	2 599	44.5		94 921	35.5
Not stated	33 024	12.6	413	7.1		33 437	12.5
Maternal BMI					***<0.001***		
< 18.5 kg/m^2	13513	5.2	233	4.0		13 746	5.1
18.5–24.9 kg/m^2	180295	68.8	3891	66.7		184 186	68.8
25–29.9 kg/m^2	46616	17.8	1137	19.5		47 753	17.8
30 kg/m^2 ≥	21362	8.2	574	9.8		21 936	8.2
Missing data	95	0.0	2	0.0		97	0,0
Parity					***<0.001***		
0	127684	48.8	4345	74.4		132 029	49.3
1	97868	37.4	1353	23.2		99 221	37.1
2≥	36329	13.9	139	2.4		36 468	13.6
Smoking during pregnancy					***<0.001***		
No	233049	89.0	5368	92.0		238 417	89.1
Yes	28832	11.0	469	8.0		29 301	10.9
Cervical excision procedure					***<0.001***		
No	255164	97.4	5566	95.4		260 730	97.4
Cold-knife	2570	1.0	119	2.0		2 689	1.0
Other – LLETZ	4147	1.6	152	2.6		4 299	1.6
Resection of uterine septum					***<0.001***		
No	252770	96.5	4753	81.4		257 523	96.2
Yes	9111	3.5	1084	18.6		10 195	3.8
Other uterine malformation					***<0.001***		
No	260118	99.3	5744	98.4		265 862	99.3
Yes	1763	0.7	93	1.6		1 856	0.7
Bleeding in pregnancy					***<0.001***		
No	243991	93.2	5035	86.3		249 026	93.0
Yes	17890	6.8	802	13.7		18 692	7.0
Preeclampsia / HELLP					***<0.001***		
No	257528	98.3	5685	97.4		263 213	98.3
Yes	4353	1.7	152	2.6		4 505	1.7
Maternal heart, renal or pulmonary illness					*0,181*		
No	257136	98.2	5745	98.4		262 881	98.2
Yes	4745	1.8	92	1.6		4 837	1.8
Gestational age at birth (weeks)					***<0.001***		
< 28	761	0.3	56	1.0		817	0.3
28 to 31 and 6/7	950	0.4	47	0.8		997	0.4
32 to 33 and 6/7	1229	0.5	48	0.8		1 277	0.5
34 to 36 and 6/7	8216	3.1	298	5.1		8 514	3.2
37 ≥	250725	95.7	5388	92.3		256 113	95.7

BMI: body mass index.
LLETZ: large loop excision of transformation zone.
HELLP syndrome: syndrome with hemolysis, elevated liver enzymes, and low platelet count.

Table 2: Unadjusted and adjusted∗ odds ratio (OR) for spontaneous preterm birth at different gestations for women conceiving after IVF/ICSI compared to women conceiving spontaneously, Slovenia, 2002–2015.

Gestation (weeks)	Unadjusted odds ratio	Confidence interval	p value	Adjusted∗ odds ratio	Confidence interval	p value
< 32	2.801	2.292 – 3.424	< 0.001	1,555	1.256 – 1.925	< 0.001
32 to 36 and 6/7	1.705	1.526 – 1.904	< 0.001	1,300	1.159 – 1,459	< 0.001

∗ Adjusted for 11 covariates: maternal age, maternal education, maternal BMI, parity, smoking during pregnancy, history of cervical excision procedure, history of hysteroscopic resection of uterine septum, presence of other congenital uterine malformations, bleeding in pregnancy, preeclampsia or HELLP in pregnancy and maternal heart, and pulmonary or renal illness.

A total of 11 605 singleton births (4.3%) in our population were premature, before 37 weeks of gestation. The distributions of preterm births according to gestation and mode of conception are also shown in Table 1.

We have calculated the unadjusted odds ratios (OR) for very preterm birth, before 32 weeks of gestation and for moderate preterm birth, between 32 and 36 6/7 of gestation, for women, who conceived after IVF-ICSI, compared to women conceiving spontaneously. Unadjusted OR for very preterm birth in pregnancies after IVF-ICSI conception was 2.8, for moderately preterm birth 1.7. After adjusting for included known confounders, the OR remained statistically significantly elevated. The results are presented in Table 2.

We also prepared multivariate analysis of different factors contributing to premature birth in our study population taking into account twelve covariates: mode of conception, maternal age, maternal education, maternal BMI, parity, smoking during pregnancy, history of cervical excision procedure, history of hysteroscopic resection of uterine septum, presence or previous surgical correction of other congenital uterine malformation, bleeding in pregnancy, preeclampsia or HELLP in pregnancy and a history of maternal heart, and renal or pulmonary illness. The risk factors for very preterm birth, before 32 weeks, and moderate preterm birth, between 32 and 36 6/7, are presented in Table 3.

According to this multivariate analysis, there are some factors contributing to the risk for very preterm birth in our population. Those factors are age more than 35 years, being underweight or obese, not having any professional education, smoking during pregnancy, first birth, bleeding, preeclampsia or HELLP during this pregnancy, and pregnancy after IVF/ICSI, which all have OR between 1.4 and 2.1. Similarly, factors for moderate preterm birth between 32 and 36 6/7 having OR between 1.4 and 2.1 are age more than 40 years, being underweight, first birth, LLETZ, and bleeding in pregnancy.

The most important risk factors for very preterm birth, which have OR higher than 2.4, are having a history of any cervical excision procedure, having previous hysteroscopic resection of uterine septum, having or being previously operated for other congenital uterine malformation, or bleeding any time in pregnancy. The most important risk factors for moderate preterm birth which have OR higher than 2.4, are having a history of any cervical excision procedure, having previous hysteroscopic resection of uterine septum, and having or being previously operated for other congenital uterine malformation.

4. Discussion

4.1. Principal Findings. Our results showed that the risk of sPTB in singleton pregnancies after IVF/ICSI is significantly greater than that in spontaneously conceived singletons. Our findings are in agreement with the most recent meta-analysis of Cavoretto et al. [17]. In this meta-analysis it has been stated that the findings should be interpreted with caution given the low quality of the available evidence. Therefore, our results could be of importance for clinicians to increase their surveillance in these patients.

As presented in Table 1, there are some important differences between women giving birth after IVF/ICSI conception and spontaneous conception. In the IVF/ICSI group the proportion of women under 30 years was three times lower (18.6% versus 52.5%; $p < 0.001$). Similarly, there were substantially more women aged 35 and more in the IVF/ICSI group (38.6% versus 14.1% $p < 0.001$).

For women, who conceive after IVF/ICSI, it is significantly more likely that they are nulliparous and have significantly more cervical excision procedures, significantly more resections of uterine septum, and significantly more other congenital malformations than women who conceive spontaneously. Furthermore, they are more likely to bleed at any time in pregnancy and to have other complications such as preeclampsia and HELLP than women, who conceive spontaneously. Therefore we performed an adjusted OR analysis. In this analysis, the OR for very preterm and moderate preterm birth remained significantly higher, which implies that IVF/ICSI might be an independent risk factor for preterm birth. Underweight patients in our analysis have higher OR for preterm birth than overweight or obese patients, which has been described before [18]. Regarding other included covariates in the analysis we have found that history of cervical excision procedure and history of uterine anomaly or hysteroscopic resection of uterine septum had an important increased OR for preterm birth.

Cold knife conisation and LLETZ were already shown to be important independent risk factors for premature birth in our recent population-based cohort study [14] and in some other studies [19–21]. Previous studies [3–9, 12] have shown that IVF treatment also increases the risk for preterm birth. If a woman has a history of surgical treatment for CIN combined with IVF, the risk for preterm birth was shown to be three times higher in study by Jakobsson et al. [11]. Similar results were obtained also with our present population-based study. Nevertheless, if a woman has high-grade squamous intraepithelial lesion, it has to be treated

Table 3: Multivariate analysis of risk factors for spontaneous preterm birth before 32 weeks and between 32 and 36 6/7 weeks of gestation, Slovenia, 2002–2015.

Preterm birth	before 32 weeks				between 32 to 36 and 6/7 weeks			
	Odds ratio	95% confidence interval		p value	Odds ratio	95% confidence interval		p value
Covariates		Lower	Upper			Lower	Upper	
Maternal age < 25								
25–29 years	1,025	0,878	1,196	0,758	1,031	0,966	1,099	0,358
30–35 years	1,127	0,956	1,329	0,153	1,106	1,032	1,185	0,005
35–39 years	1,496	1,237	1,809	< 0,001	1,309	1,204	1,424	< 0,001
40 ≥	1,975	1,491	2,617	< 0,001	1,594	1,390	1,828	< 0,001
Maternal education: Tertiary								
Secondary or professional	1,232	1,098	1,382	<0,001	1,074	1,020	1,13	0,006
Vocational	1,282	1,102	1,491	0,001	1,148	1,073	1,228	< 0,001
Primary or less	1,801	1,456	2,227	< 0,001	1,423	1,289	1,572	< 0,001
Not stated	0,712	0,588	0,861	< 0,001	1,077	1,005	1,154	0,037
Second birth								
First birth	1,443	1,293	1,611	<0,001	1,426	1,36	1,496	< 0,001
Third birth or more	1,143	0,979	1,335	0,092	1,093	1,019	1,171	0,012
Smoking during pregnancy	1,445	1,265	1,650	<0,001	1,313	1,237	1,395	< 0,001
BMI 18.5 – 24.9 kg/m^2								
BMI < 18.5 kg/m^2	1,604	1,341	1,917	<0,001	1,456	1,346	1,576	< 0,001
BMI 25 – 29.9 kg/m^2	1,021	0,9	1,158	0,749	0,935	0,884	0,989	0,018
BMI 30 kg/m^2 ≥	1,207	1,025	1,422	0,024	0,918	0,849	0,994	0,034
Heart, renal, pulmonary illness	1,255	0,928	1,698	0,141	1,135	0,986	1,308	0,078
Pregnant after IVF/ICSI	1,555	1,256	1,925	< 0,001	1,300	1,159	1,459	< 0,001
No cervical excision procedure								
Cold knife conization	6,162	5,024	7,557	< 0,001	2,455	2,137	2,821	< 0,001
Other – LLETZ	2,735	2,162	3,460	< 0,001	1,770	1,56	2,007	< 0,001
Resection of uterine septum	2,858	2,454	3,328	< 0,001	1,369	1,249	1,500	< 0,001
Other uterine malformation	2,401	1,690	3,413	< 0,001	2,404	2,043	2,828	< 0,001
Preeclampsia / HELLP	1,595	1,199	2,122	0,001	1,316	1,144	1,513	< 0,001
Bleeding in pregnancy	3,078	2,734	3,465	<0,001	1,853	1,74	1,974	< 0,001

regardless of pregnancy planning, in order to prevent the development of cervical cancer, whereas low-grade squamous intraepithelial lesions can be left untreated and followed up by colposcopy. It has been shown that the risk for spontaneous abortion is decreased, if pregnancy occurs more than 12 months after the treatment procedure for cervical precancerous lesion, whereas the risk for preterm birth was not affected by this time interval [22, 23].

Hysteroscopic resection of uterine septum was performed in 18.6% women (n = 1084) after IVF/ICSI and in only 3.5% of women (n = 9 111) conceiving spontaneously ($p < 0.001$). This is the consequence of diagnostic and operative interventions to find the exact cause of infertility before sending the infertile couple to IVF/ICSI. In Slovenia, we tend to have a holistic approach to infertility and we try to treat it in causative manner. Therefore, if we do not find a clear cause of infertility, such as severe teratozoospermia or blocked fallopian tubes, we perform diagnostic hysteroscopy and laparoscopy [24]. If a uterine septum is found during hysteroscopy, we perform a resection at the same time. Furthermore, we perform a diagnostic hysteroscopy also after several embryo transfers of top quality embryos without pregnancy.

Those are the reasons for such a big discrepancy between the two groups of women. It is known that the presence of untreated uterine anomalies is connected to preterm birth [25]. The hysteroscopic surgery of subseptate or septate uterus itself may not restore all aspects of uterine performance, but, according to the literature, it helps to reduce the risk of pregnancy loss and preterm birth [25–28], whether the hysteroscopy plays solely circumstantial or sometimes causative role in preterm birth remains unclear. Since the cervical canal has to be dilated at hysteroscopy, smaller diameter instruments in combination with prostaglandin cervical priming are being used currently [29].

Due to these findings, the procedures on cervix and uterus should be clearly indicated, especially in infertile population, since they might change integrity of the cervix in subsequent pregnancy.

Bleeding any time in pregnancy was also more common in the group of women after IVF/ICSI. It is already known

that bleeding and spontaneous abortion in first trimester occur more common after IVF/ICSI than after spontaneous conception. To some extent, this can be contributed to higher aneuploidy rate due to the advanced age of both partners, mostly female, but also male partner [30, 31]. On the other hand, the surplus transferred and implanted embryos could be degraded, which is known as "the vanishing twin syndrome". There is substantial evidence that very preterm birth is connected to vanishing of one gestational sac [32].

Placenta previa and certain other placental abnormalities, followed by antepartum hemorrhage, generally occur more often in pregnancies after IVF and the causes are still obscure [33–36]. Other placentation abnormalities, probably connected with pregnancies after IVF, are placenta accreta [37, 38], vasa previa [39, 40], and abnormal umbilical cord insertion [41]. All these facts have been clearly visible also in our study, where bleeding in any trimester of pregnancy was twice more common after IVF/ICSI than in spontaneous pregnancies.

Preeclampsia is also more common in pregnancies after IVF, mostly due to infertile population attributes, since after adjustment for the confounders the association was weak [42].

4.2. Meaning of the Findings, Clinical Implications. This study confirms findings that the population of women, who conceive after IVF/ICSI, is different than the population of women, conceiving spontaneously, and they deserve a closer and more dedicated follow-up during their long wanted pregnancy.

Furthermore, in this large cross-sectional study we proposed a combination of risk factors that define population of women with highest risk for spontaneous onset of preterm delivery.

4.3. Research Implications. According to the findings, in IVF patients, the cervical neoplasia treatment methods and hysteroscopic operation techniques should have been analyzed further, to narrow groups with the highest preterm birth risk. The cervical ostium assessment prior to hysteroscopy could be of some importance.

Besides, different IVF treatment methods should have been analysed, since the perinatal outcome is not the same in all cases: children, born after fresh ET, are at higher risk for low birthweight and premature delivery as children, born after frozen-thawed ET [43, 44].

In our study no late abortions with stillborn fetuses under 500 g and before 22 gestational weeks were included. This data could possibly show another distribution of risk factors.

There are still numerous known preterm birth risk factors, including previous preterm birth, asymptomatic bacteriuria, sexual intercourse, and psychiatric disorders, which have not been analyzed or added to our model and could add an insight to the etiology of preterm birth.

4.4. Strength and Weaknesses. Our study was designed as a population-based cohort study. The main advantage of a population-based study over 14 years is the large number of births available for analysis and inclusion of all population subgroups (by social class, by region, by life-style, by religion, etc.). In Slovenia, there is a universal access to assisted reproductive procedures, regardless of social status. The costs for six cycles of IVF for the first child and four cycles for the second or third child are covered by medical insurance for every infertile couple, where another treatment of infertility was not successful or not possible. This fact and total population inclusion diminishes the existence of selection bias in our study.

We realize that by employing data from administrative sources one could question the quality of such data. As described, the data source is obligatory by law, is in use for more than 30 years in Slovenia, is predefined, has regular quality checks, and is made of data gathered in medical records which are produced by medical staff in maternity hospitals; this is why we consider our data to be of reasonably good quality.

One of the weaknesses of our study that was already mentioned is the fact that no late abortions were included in the analysis.

5. Conclusion

In this large cross-sectional national study we proposed a combination of risk factors that define population of women with higher risk for preterm birth. We found that even after adjustment IVF/ICSI represents a single risk factor for early and late spontaneous preterm birth even after adjustment with other risks such as maternal age, smoking, or a history of invasive procedures for either cervical intraepithelial neoplasia or infertility. Therefore, these women deserve a more close and dedicated follow-up during their pregnancy.

Data Availability

The data from Medical Birth Registry, the National Perinatal Information System of Slovenia, was used to support the findings of this study. These administratively collected anonymous entries of data on Slovenian residents have not been made available since they are protected by the law.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Assessment of Uterocervical Angle Width as a Predictive Factor of Preterm Birth: A Systematic Review of the Literature

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Background. Uterocervical angle (UCA) has been recently proposed as a potential marker that could accurately predict preterm birth (PTB). The purpose of the present systematic review is to accumulate current evidence and provide directions for future research. *Materials and Methods*. We used the Medline (1966–2018), Scopus (2004–2018), Clinicaltrials.gov (2008–2018), EMBASE (1980-2018), Cochrane Central Register of Controlled Trials CENTRAL (1999-2018), and Google Scholar (2004-2018) databases in our search. *Results*. Eleven studies were finally included in the present systematic review that evaluated data from 3,018 women. The significant heterogeneity in terms of outcome reporting and outcome reporting measures (use of optimal cut-off values) precluded meta-analysis. However, existing data support that second trimester UCA measurement might be used as a predictive factor of PTB <34 weeks, as at least two studies in unselected singleton pregnancies and two studies in pregnancies with an ultrasonographically shortened cervix seem to support this hypothesis. The most commonly reported cut-off values were 105° and 95°. *Conclusions*. UCA measurement during the second trimester of pregnancy may be a useful method of determining women at risk of delivering preterm. However, more studies are needed to assess the reproducibility of these findings and reach conclusive evidence.

1. Introduction

Preterm birth (PTB) is a leading cause of perinatal morbidity and mortality and is estimated to complicate approximately 10-12% of pregnancies [1]. To date, the optimal strategy of pregnancies at risk of preterm birth remains unclear. Progesterone, cervical cerclage, and the Arabin pessary have been used as potential management strategies in women with singleton pregnancies with a short cervix and history of previous spontaneous preterm birth [2]. In a recent network meta-analysis Jarde et al. observed that progesterone seems to be the best intervention; however, the significant heterogeneity of included studies precluded safe interpretation of their findings [3]. Screening of pregnancies remains also problematic as the majority of current strategies is far from an optimal diagnostic accuracy. Fetal fibronectin has been suggested as a potential biomarker for the prevention of preterm birth; however, its sensitivity is relatively low (34%) [4]. Current data also suggest that cervical length (CL) measurement may help identify these women as it may accurately predict pregnancies at risk of preterm birth [5, 6]. In this line, current guidelines suggest that women with a history of spontaneous preterm delivery or second trimester loss, as well as those with a short cervix (<25 mm) in a transvaginal ultrasound scan between 16 and 24 weeks of gestation should be offered treatment with cerclage or progesterone [7].

Uterocervical angle (UCA) represents a novel ultrasonographic marker that is defined as the triangular segment measured between the lower uterine segment and the cervical canal. It is measured using a line that starts from the internal cervical os (that is extended along the cervical canal) and a second line that tracks the internal segment of the anterior uterine wall. During the last years several studies investigated the potential impact of UCA for the prediction of preterm

birth. The rationale behind the hypothesis of this association is based on the potential mechanical properties of this angle, which seems to act as a preventive barrier when it is acute. The first article that supported this assumption was written by Cannie et al. who supported that the efficacy of the Arabin pessary in preventing preterm birth was significantly influenced by the change in the UCA pre- and postpessary insertion [8]. Keepanasseril et al. also suggested that UCA may be a mechanical barrier that might influence the progress of labour [9]. These authors supported at 2007 that a posterior cervical angle of at least 100° is accompanied by a specificity and specificity of 65% and 72%, respectively, for the prediction of successful induction of labour in nulliparous women. Assuming that this angle might be also predictive in determining women at risk for preterm birth a significant number of articles were published. The purpose of the present systematic review is to accumulate and present current evidence in this field and to provide recommendations for future research.

2. Materials and Methods

The present systematic review was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [10].

2.1. Information Sources and Search Methods. We used the Medline (1966–2018), Scopus (2004–2018), Clinicaltrials.gov (2008–2018), EMBASE (1980-2018), Cochrane Central Register of Controlled Trials CENTRAL (1999-2018), and Google Scholar (2004-2018) databases in our primary search along with the reference lists of electronically retrieved full-text papers. The date of our last search was set at 28 February 2018. Our search strategy included the text words "*angle, preterm, cervix, cervical*" and is schematically presented in the PRISMA flow diagram (Figure 1).

The studies were selected in three consecutive stages. Following deduplication, the titles and abstracts of all electronic articles were screened by two authors (V. P. and G. D.) to assess their eligibility. The decision for inclusion of studies in the present systematic review was taken after retrieving and reviewing the full text of articles that were held potentially eligible. Potential discrepancies in this latter stage were resolved by the consensus of all authors.

2.2. Quality and Risk of Bias Assessment. The risk of bias and methodological quality of the included studies was explored using the Newcastle-Ottawa Scale (NOS), which evaluates the selection of the study groups, the comparability of the groups, and the ascertainment of the exposure or outcome of interest [11].

2.3. Study Selection

2.3.1. Types of Studies and Patients. The eligibility criteria for the inclusion of studies were predetermined. No language restrictions were applied. All observational studies as well as randomized trials that assessed the differences and, whenever present, the predictive accuracy of UCA in preterm birth were held eligible for inclusion (irrespective of the existence of other variables from the patients' history including preterm premature rupture of membranes (PPROM), previous preterm births, parity, gravidity, and singleton/multiple gestation). Conference abstracts were also included. Case reports as well as experimental animal studies and reviews were not included in the qualitative analysis.

2.3.2. Outcome Measures. The mean difference in uterocervical angle among pregnancies delivered at term and preterm (<37 weeks of gestation) was predefined as primary outcome measure. The sensitivity and specificity of UCA in detecting pregnancies at risk of delivering prior to the 37th, 34th, 32nd, and 28th week of gestation were also defined as primary outcome measures.

Secondary outcome measures were defined following completion of data extraction and included differences in gestational latency period following PPROM and latency period following cerclage placement.

3. Results

Eleven studies were finally included in the present systematic review that evaluated data from 3,018 women (Table 1) [12–22]. Among them, 5 studies evaluated pregnancy outcomes in unselected singleton pregnancies [14, 16, 17, 21, 22], two studies reported outcomes in women with a shortened cervix that were offered cerclage [6, 21], one study evaluated changes in UCA in women with a shortened cervix that were followed up with at least two measurements of cervical length during the second trimester of pregnancy [15], two studies enrolled unselected twin pregnancies [13, 18], and one study evaluated the impact of UCA in the latency period of pregnancies complicated by PPROM [20]. The results of the Newcastle-Ottawa Scale are presented in Table 2. In general, the quality of most studies was evaluated as fair–high; however, their comparability was evaluated as inappropriate as none of the included cohorts presented adjusted analysis according to the cervical angle (a factor that has already been described as predisposing for preterm birth). Only one study evaluated the role of confounders other than cervical length (including maternal age, nulliparity, race, and obesity) on uterocervical angle [22]. The single case control study that was included in the present systematic review scored three stars for patient selection, no stars for comparability, and three stars for exposure [21].

3.1. Outcomes in Unselected Singleton Gestations. Sur et al. found significant differences in mean UCA between pregnancies that delivered preterm (<37 weeks) and those that delivered at term, during both the first (114.2° versus 93.0°) and second trimester of pregnancy (127.66° versus 103.65°) [14]. In line with this observation was the study of Faras-Llobet et al. that suggested that the angle during the second trimester screening was significantly wider in women that delivered at <34 weeks compared to those that delivered at term (105.16° versus 94.53°) [5]. In another

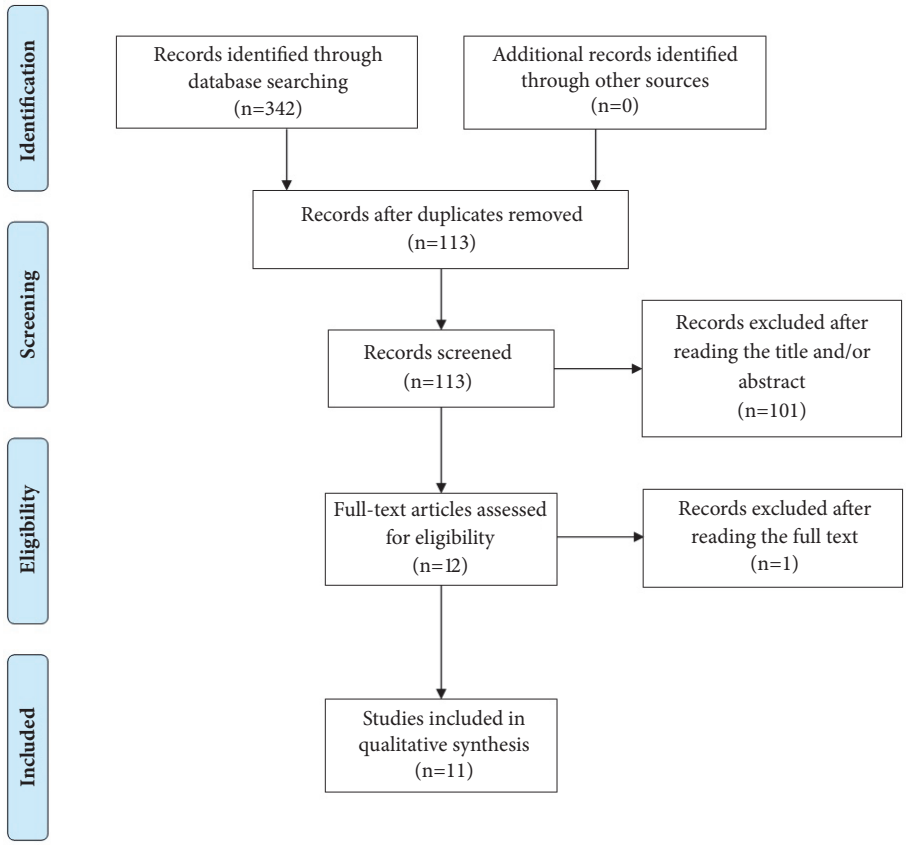

Figure 1: Search plot diagram.

study, however, the same group of researchers reported there is a minimal difference among women that delivered at term compared to those that delivered preterm (<37 weeks) (103.6° versus 101.7°), implying that at least second trimester measurement may not be as predictive as we would like to think so [13]. Martinez et al. also confirmed that pregnant women with a wide UCA are prone to deliver preterm (<34 weeks) compared to women that delivered at term (106.1° versus 99.5°) [21]. They also mentioned that UCA was independent of the CL measurement and could thus be used in predictive models combined with CL expressed as multiples of the median (MoM), maternal characteristics, and history (maternal age and history of previous PTB). Finally, Dziadosz et al. observed that second trimester UCA measurement could detect the possibility of preterm birth <37 weeks with a sensitivity of 80% when the angle was ≥95° and <34 weeks with a sensitivity of 81% when the angle was ≥105° [22]. The same authors also reported that when they performed stepwise linear regression analysis they observed that UCA was dependent on maternal age, obesity at conception, nulliparity, and race.

3.2. Outcomes in Pregnancies with an Ultrasonographically Shortened Cervix. Swanson et al. investigated the accuracy of UCA in predicting gestational latency in women with physical examination indicated cerclage [12]. They used predetermined cut-off values of UCA at 95° and 105° to stratify patients and used ultrasound images that were obtained prior to cerclage placement. No differences were noted and the authors concluded that UCA cannot predict gestational latency in women undergoing physical examination indicated cerclage. On the other hand, Knight et al. suggested that UCA angle prior to delivery was predictive of cerclage failure [19]. However, this was not the case with UCA angle estimation prior to cerclage placement or shortly after cerclage placement. The authors used optimal cut-offs to estimate the potentially predictive accuracy of the method and reported that a cut-off of 108° was able to detect delivery prior to 34 weeks with a sensitivity of 97% and specificity of 65%. Concerning delivery prior to 28 weeks the optimal performance of the ROC analysis was observed at 112° with a sensitivity of 100% and a specificity of 62%. Finally, Lynch et al. evaluated women that were sequentially screened during the second trimester of pregnancy and observed that the difference in UCA among these measurements was not able to predict preterm birth [15]. However, they did mention that a final UCA of ≥105° prior to 25 weeks of gestation was associated with an increased risk of preterm birth <34 weeks (24.2% versus 6.8%, $p=.01$).

3.3. Outcomes in Twin Pregnancies. As previously mentioned only two studies reported outcomes in unselected twin

Table 1: Methodological characteristics and patient selection in included studies (PEIC, physical examination indicated cerclage; PPROM, preterm premature rupture of the membranes).

Study characteristics				
Year; author	***Study design***	***Patient n***	***Inclusion criteria***	***Outcomes of interest***
2018; Swanson	Retrospective cohort	60	Ultrasound examination no more than 3 weeks prior to PEIC (length <2cm and dilatation)	Gestational latency period
2018; Lynch	Retrospective cohort	137	Unselected twin pregnancies that had an ultrasound scan between 14 and 25 weeks.	Spontaneous preterm birth (<37 weeks)
2017; Sur	Prospective cohort	100	Women with singleton uncomplicated pregnancy scanned during the 1st and 2nd trimester	Spontaneous preterm birth (<37 weeks)
2017; Lynch	Retrospective cohort	176	Women with singleton pregnancy and CL<25mm between 14 and 25 weeks. Women with only 1 measurement of CL were excluded	Rates of spontaneous preterm birth (<37 weeks) in women with a short cervix
2017; Farras Llobet poster	Prospective study	499	Unselected singleton pregnancies that had an ultrasound scan between 18 and 24 weeks.	Spontaneous preterm birth (<37 weeks)
2017; Farras Llobet	Prospective case control	275	Unselected singleton pregnancies that had an ultrasound scan between 18 and 24 weeks.	Spontaneous preterm birth (<34 weeks)
2017; Knight (2)	Retrospective cohort	259	Twin pregnancies that had an ultrasound scan between 16 and 23 weeks.	Spontaneous preterm birth (<32 weeks and <28 weeks)
2017; Knight	Retrospective cohort	142	Women with PEIC that had ultrasound examination 1 week after cerclage placement	Preterm birth (<34 weeks, <28 weeks)
2017; Kathir	Prospective cohort	80	Women with singleton pregnancy between 28 and 34 weeks, PPROM, not in labour	Pregnancy latency period
2016; Martinez	Retrospective nested case control	318	Unselected singleton pregnancies that had an ultrasound scan between 14 and 24 weeks.	Spontaneous preterm birth (<34weeks)
2016; Dziadosz	Retrospective cohort	972	Women with singleton pregnancy that had an ultrasound scan between 16 and 24 weeks	Spontaneous preterm birth (<37 weeks and <34 weeks)

pregnancies that were ultrasonographically evaluated during the second trimester. Lynch et al. used predetermined cut-off values of 95° and 105° and observed that they were both associated with an increased risk of PTB <37 weeks ((55.9% versus 31.6%, $p=.05$ and 58.3% versus 35.3%, $p=.02$, respectively) [13]. The authors also compared performance metrics of UCA with CL and observed that UCA was accompanied by significant sensitivity (exceeding 80%) but low specificity (less than 35%), whereas CL was more specific (98.5%) but less sensitive (12.5%). Knight et al. also evaluated second trimester UCA and observed that the use of optimal cut-offs resulted in enhanced predictive accuracy compared to CL measurement (<20mm) for PTB <34 and <28 weeks [18]. Specifically, the cut-off of 110° was accompanied by 80% sensitivity and 82% specificity for the prediction of PTB <34 weeks.

Table 2: Newcastle-Ottawa scale score of included studies.

Date; Author	Newcastle-Ottawa Assessment Scale								
	Selection				Comparability	Outcome			Total
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study		Assessment of outcome	Adequacy of duration of follow up	Adequacy of completeness of follow up	
2018; Swanson	-	★	★	★	-	★	★	★	6
2018; Lynch	★	★	?	?	-	?	★	★	4
2017; Sur	★	★	★	★	-	★	★	★	7
2017; Lynch	★	★	★	★	-	★	★	★	7
2017; Farras Llobet poster	★	★	★	★	-	?	★	★	6
2017; Farras Llobet	-	★	★	★	-	★	★	★	6
2017; Knight (2)	★	★	★	★	-	★	★	★	7
2017; Knight	★	★	★	★	-	★	★	★	7
2017; Kathir	★	★	★	★	-	★	★	★	7
2016; Dziadosz	★	★	★	★	★	★	★	★	8

3.4. Outcomes from Other Published Studies. A study was published at 2017 by Kathir et al. that investigated whether CL and UCA were associated with gestational latency in women with PPROM [20]. The authors reported that whereas CL did not influence the latency period in these cases UCA exerted a mild effect that requires further investigation in the future (Hazard ratio 1.03, 95% CI 1.01 – 1.06, $p=.003$).

4. Discussion

Existing data support that second trimester UCA measurement might be used as a predictive factor of PTB <34 weeks, given that at least two studies in unselected singleton pregnancies, and two studies in pregnancies with an ultrasonographically shortened cervix seem to support this hypothesis. However, to date most of the available evidence is based in optimal cut-off values; hence the accumulation of data in a meta-analytic approach remains out of the question.

The actual factors that influence this angle, however, remain, to date, unknown. Preconceptional UCA is directly related to uterine version and flexion and this factor should be evaluated in large future cohorts. Constitutional changes in the physiology of the cervix during pregnancy may also affect the flexibility of the cervix and significantly modify UCA. At 2012 Heller et al. evaluated uterine cervices from 22 cases of obstetric hysterectomy and observed that the mean percent of collagen was significantly higher in cervices on nonpregnant uteri compared to pregnant uteri (73.5±3.5% versus 21.5±2.2%) [23]. This study indicates the presence of significant differences in the physiology of the cervix during pregnancy. Recently, Sundtoft et al. also suggested that women with cervical insufficiency have lower collagen concentrations (63.5 ± 5.1%) compared with controls (68.2 ± 5.4%) $p<.001$ [24]. None of the existing studies evaluates directly the impact of the cervical microenvironment on the UCA. However, a recent indirect comparison between collagen fiber orientation and dispersion in the upper cervix of pregnant and nonpregnant women suggested that collagen fiber dispersion and direction may influence cervical remodeling during pregnancy [25].

The main strength of the present systematic review is the accumulation for the first time in the international literature of evidence related to the diagnostic accuracy of UCA for the prediction of preterm birth. The majority of included studies scored high for patient selection and outcome reporting; hence current data can be considered for the conduct of future research in this field. On the other hand, the wide heterogeneity in terms of the selected population, outcome reporting (UCA cut-off value), and outcome of interest (gestational week that was used as a cut-off value of preterm birth) among the included studies rendered impossible the conduct of a meta-analysis of diagnostic accuracy (Table 1).

Taking this information into consideration and despite the potential pathophysiological background that was already mentioned, existing evidence, although promising, does not suffice to introduce UCA in current clinical practice as a predictive factor that may be used for decision-making regarding management of women at risk of delivering preterm. This is why, future studies are needed to evaluate the diagnostic accuracy of this index, and these should specifically consider the use of cut-off values and outcomes of interest (preterm birth rates based on specific gestational weeks) that were presented in the present systematic review. Moreover, they should adjust their findings according to the CL as it remains

unknown whether an overlap between CL and UCA exists that might influence the detection rate of the latter index.

Disclosure

The present systematic review is based on previously published data.

Conflicts of Interest

The authors report no conflicts of interest.

Authors' Contributions

Georgios Daskalakis conceived the idea, conducted the electronic search and tabulated data, Mariana Theodora MD, Panagiotis Antsaklis, Michail Sindos, Themos Grigoriadis performed the data collection and wrote the manuscript, Aris Antsaklis and Nikolaos Papantoniou wrote and revised the manuscript, Dimitrios Loutradis wrote and critically revised the manuscript and Vasilios Pergialiotis formed the tables, conducted the electronic search and tabulated data.

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Research Article

A Retrospective Study on the Risk of Respiratory Distress Syndrome in Singleton Pregnancies with Preterm Premature Rupture of Membranes between 24+0 and 36+6 Weeks, Using Regression Analysis for Various Factors

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Aim. This study aimed to investigate the cause of respiratory distress syndrome (RDS) in neonates from singleton pregnancies with preterm premature rupture of membranes (pPROM) between 24+0 and 36+6 weeks by using regression analysis for various factors. *Methods.* In 175 singleton pregnancies with pPROM, 95 cases of RDS (54,29%) were diagnosed. In all cases the following information was collected: latency period of PROM, gestational age at birth, Umbilical Artery Pulsatility Index (UA PI), Middle Cerebral Artery Pulsatility Index (MCA PI), fetal distress, antenatal steroids use, delivery type, pregnancy hypertension disease, gestational glucose intolerance or diabetes, neonatal laboratory parameters, gender, weight, Apgar score, and other neonatal complications. Logistic regression analysis was used to investigate the effect of variables on RDS. *Results.* The results of logistic regression analysis showed that the following variables are closely correlated with RDS: female gender (OR=0.52; 95%CI:0.28-0,97), antenatal steroids use (OR=0,46; 95%CI:0,34-0,64), abnormal UA PI and MCA PI (OR=2.96; 95%CI:1,43-6,12) (OR=2.05; 95%CI:1,07-3,95), fetal distress (OR=2.33; 95%CI:1,16-4,71), maternal HGB (OR=0.69; 95%CI:0,5-0,96), and neonatal RBC, HGB (OR=0.32; 95%CI:0,19-0,55) (OR=0.75; 95%CI:0,65-0,88). *Conclusions.* The main RDS risk factors in premature neonates are gender, abnormal fetoplacental circulation, and fetal distress. The laboratory parameters such as lower RBC and HGB count are observed in infants with RDS.

1. Introduction

Premature rupture of membranes (PROM) occurs in approximately 3-10% of all pregnancies; it is defined as a rupture of the membranes an hour before the start of uterine contractions, regardless of gestational age [1, 2]. Taking into account the gestational age, PROM is divided into two categories: before the 37th week of pregnancy defined as preterm premature rupture of membranes (pPROM) and after the 37th week of pregnancy referred to as term premature rupture of membranes (tPROM). pPROM complicates approximately 2-4% of singleton pregnancies and about 7-20% of multiple pregnancies [1, 2]. This complication is a significant cause of an increased morbidity and mortality for both infants and mothers [3, 4]. pPROM occurs among 30-40% of all preterm births, which is still a significant problem in perinatal medicine [5, 6]. Besides prematurity, neonatal complications include infection, sepsis, trauma,

fetal distress, intraventricular hemorrhage, and respiratory distress syndrome [7, 8].

Respiratory distress syndrome (RDS) is one of the most common causes of neonatal respiratory failure and neonatal death. The underlying pathogenesis of RDS involves developmental immaturity of lungs, leading to inadequate pulmonary surfactant production [9]. It was previously believed that the most significant RDS factor is the prematurity. Despite many studies, the reason for the occurrence of RDS still remains unclear.

2. Objectives

This study aimed to investigate the cause of RDS in neonates from singleton pregnancies with pPROM between 24+0 and 36+6 weeks, using regression analysis for various factors, and thus provide a useful reference for its prediction.

3. Material and Methods

This investigation is a retrospective study approved by the bioethics committee of Silesian Medical University in Katowice, Poland. In the Department of Gynaecology and Obstetrics of the Municipal Hospital in Ruda Śląska from January 2011 to December 2014 a total of 175 singleton pregnancies with pPROM were hospitalized. A consecutive recruitment was used in this study.

The diagnosis of pPROM met the following criteria: (1) rupture of membranes based on the history, (2) leaking amniotic fluid found in physical examination, (2) singleton pregnancies between 24 + 0/7 and 36 + 6/7 weeks of gestation. Cases with dubious diagnosis were excluded.

In all cases the following information was collected: latency period of PROM; gestational age at birth; Umbilical Artery Pulsatility Index (UA PI); Middle Cerebral Artery Pulsatility Index (MCA PI); fetal distress; antenatal steroids use; maternal age at pregnancy, maternal haemoglobin (HGB), red blood cells (RBC), white blood cells (WBC) and platelets (PLT) count, maternal C-reactive protein (CRP) level, amniotic fluid index (AFI), and delivery mode; pregnancy hypertension disease; gestational glucose intolerance or diabetes; neonatal sex; weight; Apgar score at 1st, 3rd, 5th, and 10th minute; RBC, WBC, HGB, and PLT count; CRP level; and RDS, anaemia, congenital infection, and intraventricular haemorrhage (IVH).

In 95 cases (54,29%) RDS was diagnosed based on the following criteria: (1) acute onset; (2) representative clinical manifestations including progressive respiratory distress occurring shortly after birth, characteristic grunting respiration, retractions during inspiration, cyanosis, and reduced or absent breathing sounds; (3) typical chest x-ray findings, including hypoexpansion and diffuse, fine granular densities (grade I), air bronchograms caused by the atelectatic air sacs (grade II), ground-glass appearance (grade III), or white lungs caused by diffuse bilateral atelectasis (grade IV); (4) arterial blood gas analysis showing hypoxia, hypercapnia, and oxygen tension/fraction of inspired oxygen ratio (PaO2/FiO2) ≤ 26.7 kPa.

Other diagnostic criteria used in this study were [9–12] fetal distress as a significant abnormality in the fetal heart rate according to the result of fetal heart rate monitoring; congenital infection as fetal-neonatal infectious diseases such as pneumonia /septicemia caused by intra-amniotic infection; neonatal anaemia as HGB lower than 18 g/dl; IVH was diagnosed using transfontanel ultrasonography; all IVH grades were included in the study.

Logistic regression analysis was used to investigate the effect of variables on neonatal RDS. Univariate and multivariate logistic regression models were created. A $p<0.05$ was considered to be statistically significant.

4. Results

From 9657 deliveries in the Department of Gynaecology and Obstetrics of the Municipal Hospital in Ruda Śląska during the years 2011–2014, 175 cases (3,07%) met the pPROM criteria. RDS was diagnosed in 95 cases, which represents 54.29% of the studied group. The median latency period of pPROM was 19 hours and 48 minutes.

We found that the lower Apgar score at 1st, 3rd, 5th, and 10th minute (respectively, (OR = 0.52; 95% CI 0,4-0,68; p <0.001), (OR = 0, 46; 95% CI: 0,34-0,63; p <0.001), (OR = 0.37; 95% CI: 0,24-0,56; p <0.001), and (OR = 0.4; 95% CI: 0,26-0,6; p <0.001)); females sex (OR = 0.52; 95% CI: 0.28-0,97; p = 0.039); antenatal steroid use (OR = 0,46; 95% CI: 0,34-0,64; p <0.001); abnormal Umbilical Artery Pulsatility Index (UA PI) (OR = 2.96; 95% CI: 1,43-6,12; p = 0.003); abnormal Middle Cerebral Artery Pulsatility Index (MCA PI) (OR = 2.05; 95% CI: 1,07-3,95; p = 0.031); fetal distress (OR = 2.33; 95% CI: 1,16-4,71; p = 0.018); lower maternal HGB (OR = 0.69; 95% CI: 0,5-0,96; p = 0.025); and lower neonatal RBC and HGB (OR = 0.32; 95% CI: 0,19-0,55; p <0.001) and (OR = 0.75; 95% CI: 0,65-0,88; p <0.001) were the main risk factors of RDS in premature neonates (Table 1) (Figure 1).

A higher incidence of RDS resulted in newborns with anaemia (OR = 8; 95% CI: 3,32-19,26; p <0.001); congenital infection (OR = 4.63; 95% CI: 1,8-11,94; p =0.001); and intraventricular hemorrhage (OR = 6.55; 95% CI: 1,44-29,82; p = 0.015).

In the analysis using multivariate logistic regression model, gestational age at birth (OR = 0.93; 95% CI 0,9-0,96; p <0.001), neonatal HGB (OR = 0.77; 95% CI: 0.63-0.93; p = 0.007), and neonatal PLT (OR = 0.9912; 95% CI: 0,9857-0,9967; p = 0.002) were the risk factors of RDS in premature neonates (Table 2) (Figure 2).

In this study variables such as delivery type; maternal and fetal WBC and CRP; maternal age; AFI; pregnancy hypertension disease; gestational glucose intolerance; or diabetes were not significant risk factors for RDS (p = ns) in preterm neonates.

5. Discussion

The occurrence of PROM, regardless of gestational age, is at level of 3-10% [1, 2]; 2-18% [13–15]. pPROM complicates approximately 2-4% of singleton pregnancies and 20-40%

Table 1: Univariate logistic analysis of various factors for preterm neonatal RDS.

Risk factor	Odds ratios	95% CI	p-value	Nr. of cases
PROM latency period	1,0035	(1,0009;1,0061)	0,009	168
Gestational age at birth	0,9100	(0,88;0,94)	<0,001	170
Abnormal UA PI	2,9600	(1,43;6,12)	0,003	169
Abnormal MCA PI	2,0500	(1,07;3,95)	0,031	169
Fetal distress	2,3300	(1,16;4,71)	0,018	170
Antenatal steroids use	0,4600	(0,34;0,64)	<0,001	170
Maternal HGB	0,6900	(0,5;0,96)	0,025	153
Intraventricular hemorrhage	6,5500	(1,44;29,82)	0,015	167
Congenital infection	4,6300	(1,8;11,94)	0,001	169
Anaemia	8,0000	(3,32;19,26)	<0,001	168
Neonatal PLT	0,9916	(0,9871;0,9961)	<0,001	150
Neonatal HGB	0,7500	(0,65;0,88)	<0,001	150
Neonatal RBC	0,3200	(0,19;0,55)	<0,001	150
Gender (female)	0,5200	(0,28;0,97)	0,039	170
Apgar score at 10th min	0,4000	(0,26;0,6)	<0,001	168
Apgar score at 5th min	0,3700	(0,24;0,56)	<0,001	168
Apgar score at 3rd min	0,4600	(0,34;0,63)	<0,001	168
Apgar score at 1st min	0,5200	(0,4;0,68)	<0,001	168
Birth weight	0,9975	(0,9967;0,9983)	<0,001	170

Table 2: Multivariate logistic analysis of various factors for preterm neonatal RDS.

Risk factor	Odds ratios	95% CI	p-value	Nr. of cases
Gestational age at birth	0,9300	(0,9;0,96)	<0,001	150
Neonatal HGB	0,7700	(0,63;0,93)	0,007	150
Neonatal PLT	0,9912	(0,9857;0,9967)	0,002	150

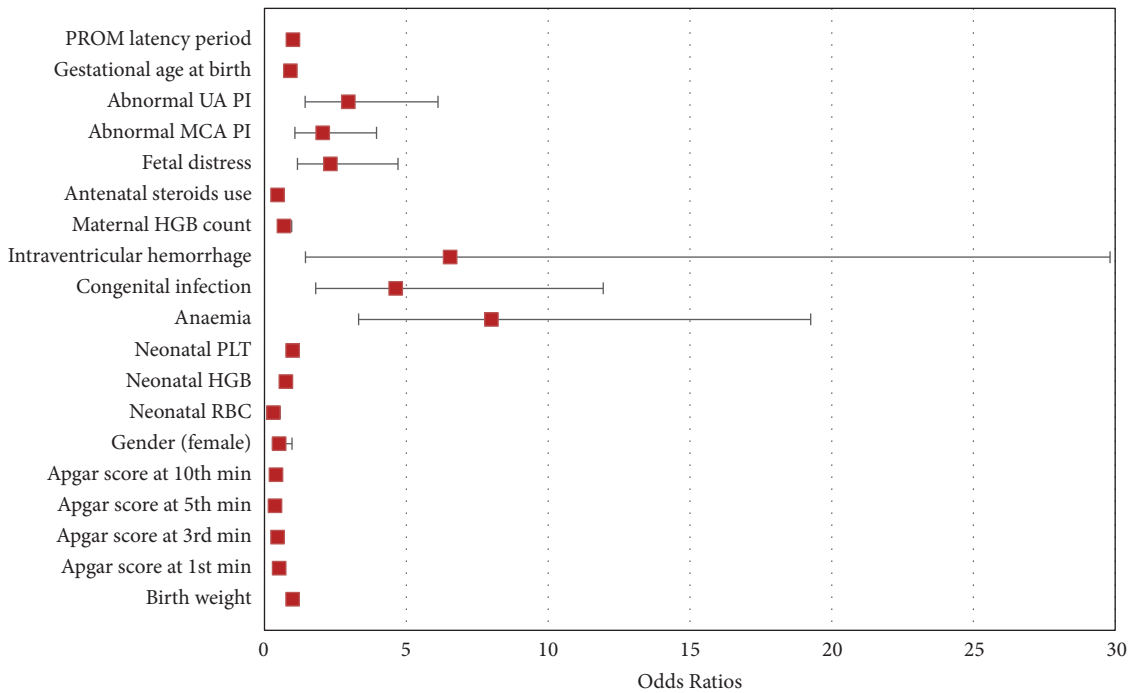

Figure 1: Odds ratios and confidence intervals for variables affecting the occurrence of preterm neonatal RDS–univariate logistic regression.

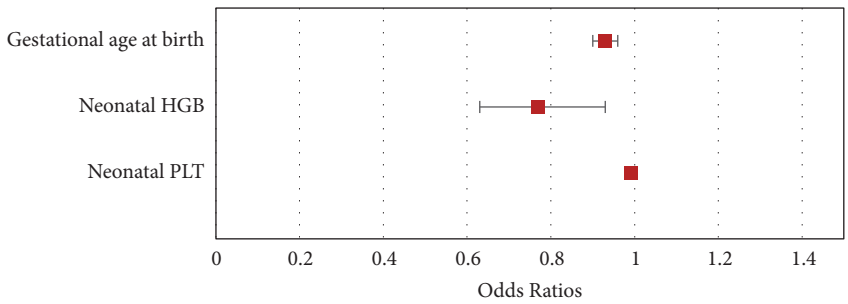

Figure 2: Odds ratios and confidence intervals for variables affecting the occurrence of preterm neonatal RDS-multivariate logistic regression.

of all PROM cases [1, 2, 8, 13, 16]. In this study pPROM frequency was 3,07% which is similar to the one given in the literature.

According to Zanardo et al., RDS developed in 55.4% of the examined newborns from pregnancies complicated by pPROM [17], whereas JoonHo LEE et al. report that, in South Korea, the RDS was diagnosed in 47% of the cases [18]. In this study, RDS amounted 54.29% which is comparable to the percentages mentioned above.

The results of this study show that gender; antenatal steroid use; abnormal UA PI and MCA PI; fetal distress; and congenital infection are the main risk factors of RDS in preterm neonates from pPROM pregnancies.

This study shows that among female gender there is lower incidence of RDS in preterm neonates. The relative risk of RDS is 0,52 times lower for females than males. These data are confirmed in the literature [9, 19–21]. It was found that in gestation the female fetal lung produces surfactant earlier than the male one. The reasons for this may be as follows: (1) androgens delay lung fibroblast secretion of fibroblast-pneumocyte factor, which can delay the development of alveolar type II cells and reduce the release of surfactant; (2) androgens slow fetal lung development by adjusting the signalling pathways of epidermal growth factor and transforming growth factor-beta; (3) estrogen promotes the synthesis of phospholipids, lecithin, and surfactant proteins A and B; and (4) estrogen also improves fetal lung development by increasing the number of alveolar type II cells and by increasing the formation of lamellated bodies [9, 22–25].

Our study confirms that antenatal steroids' use reduces the risk for RDS. This fact results in the current international recommendations of the Royal College of Obstetricians and Gynaecologists (RCOG) in dealing with various accepted dosage schemes of corticosteroids.

Neonatal breathing disorders can be caused by circulatory system diseases. The main factors in this group are congenital heart disease, pulmonary hypertension, and congestive heart failure [26, 27]. No reports were found regarding fetoplacental circulation in relation to the development of neonatal RDS. However, the abnormal UA PI, MCA PI correlates with centralization of the cardiovascular system, which after the birth is an additional risk factor for RDS on the background of cardiovascular failure. Büke et al. concluded that pulmonary artery acceleration time to ejection time ratio (PATET) is a promising noninvasive tool to predict RDS in cases of preterm deliveries [28] while Laban M et al. find that measurement of fetal lung volume (FLV) or pulmonary artery resistance index (PA-RI) can help to predict RDS in preterm fetuses [29].

The results of this study show that congenital infection and fetal distress are strong RDS factors. A similar correlation was observed in many studies [9, 18, 19, 26, 30]. Fetal distress may lead to birth asphyxia. Asphyxia together with congenital infection causes the direct injury to the fetal lungs and alveolar type II cells, decreasing the synthesis and releasing surfactant [9, 31, 32]. Fetal-neonatal lung inflammation increases the permeability of the alveolar-capillary membrane to both fluid and solutes. This results in plasma proteins entering the alveolar hypophase, which further inhibits the function of surfactant [9, 31, 32].

In this study relationship between the lower count of RBC, HGB, PLT, and RDS was found. Correct levels of RBC, HGB, and PLT vary depending on the gestational age and prematurity; i.e., the less mature the newborn is, the lower the values are [33, 34]. Another factor affecting the RBC, HGB, and PLT values was the increased percentage of newborns with IUI and prolongation of PROM latency, who are characterized by significantly lower count of RBC, HGB, and PLT compared to noninfected newborns [34, 35].

There is also higher incidence of RDS in newborns affected by other complications such as anaemia, congenital infection, and intraventricular hemorrhage. This was also reflected in the literature [2, 13, 16, 31, 36]. Furthermore, in this study the occurrence of RDS was associated with lower PLT count; its deficiency leads to bleeding. Additional PLT reduction risk factors are prematurity and intrauterine infection [33]. This leads to the occurrence of both RDS and intraventricular hemorrhage [34].

6. Conclusions

The main risk factors of RDS in premature neonates are gender, abnormal fetoplacental circulation, and fetal distress. Other neonatal complications such as anaemia, congenital infection, and intraventricular haemorrhage increase the risk of RDS coexistence. The laboratory parameters abnormalities such as lower RBC, HGB, and PLT count are observed in infants with RDS.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

The Microbiome and Preterm Birth: A Change in Paradigm with Profound Implications for Pathophysiologic Concepts and Novel Therapeutic Strategies

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Preterm birth poses a global challenge with a continuously increasing disease burden during the last decades. Advances in understanding the etiopathogenesis did not lead to a reduction of prematurely born infants so far. A balanced development of the host microbiome in early life is key for the maturation of the immune system and many other physiological functions. With the tremendous progress in new diagnostic possibilities, the contribution of microbiota changes to preterm birth and the acute and long-term sequelae of prematurity have come into the research focus. This review summarizes the latest advances in the understanding of microbiomes in the amniotic cavity and the female lower genital tract and how changes in microbiota structures contribute to preterm delivery. The exhibition of these highly vulnerable infants to the hostile environment in the neonatal intensive care unit necessarily entails the rapid colonization with a nonbalanced microbiome in a situation where the organism is still very prone and at an early stage of development. The global research efforts to decipher pathologic changes will pave the way to new pre- and postnatal therapeutic concepts.

1. Introduction

Microbiomes comprise commensal, symbiotic, and pathogenic bacteria, fungi, and viruses, which form an ecological entity and interact with themselves and with their particular host. For a long time, it has been assumed that microbiota colonization is restricted to body surfaces like skin and the gastrointestinal tract. However, it became clear in the recent years that microorganisms reside in nearly every human tissue including the mammary glands, the ovaries, the uterus, and the placenta. Thus the human body is colonized by trillions of microbial inhabitants. They constitute a

diverse and individually varying ecological community which in addition changes with age [1]. In line with this, the former theory that the amniotic cavity constitutes a sterile environment had to be abandoned. It became clear that the healthy maternofetal unit is colonized with microbes and that this is a prerequisite for immune maturation as well as metabolic and hormonal homeostasis. Fetal life and the first year of life are a life span which is critical for the development of a well-functioning immune system and maintenance of long-term health. For these reasons, negative early-life events pose a special risk to somatic and psychomotor development. Pathologic changes in microbiomes predispose or contribute to acute and chronic morbidities of every organ at any age. Furthermore, functional changes in microbiomes trigger infectious complications. In this review we present the latest insights into the structure and function of our microbiome and how pathologic changes contribute to preterm labor, premature birth, and the acute and long-term sequelae of prematurely born infants. Despite the fact that fungi and viruses are part of microbiomes, most research efforts so far clearly focused on bacterial diversity. Thus, other microbes than bacteria are mostly excluded in this review, simply because no data is available.

2. Functional Traits of the Microbiome of the Lower Urogenital Tract and the Fetomaternal Unit

The amniotic cavity has long been viewed as a sterile environment where the fetus is protected from the harmful external influences and threats. First reports emerged with the beginning of the new millennium, which questioned this hypothesis and described an intrauterine and placental microbial environment [2–4]. With the advancements in molecular techniques it became clear that the placenta, the amnion, and the fetus share large proportions of a common microbiome and that the maternal microbiome drives the development of the fetal immune system [5, 6]. The placental microbiome, under physiologic conditions, harbors nonpathogenic commensals including Firmicutes, Tenericutes, Proteobacteria, Prevotella, Neisseria, Bacteroidetes, and Fusobacteria but also potential pathogenic species like *Escherichia coli* [7]. As its detection is based on molecular techniques, the scientific discussion is ongoing whether the placental microbiome contains viable microbiota or just microbial components. Nonetheless, a regulatory function is currently assumed [8].

The vaginal microbiome resembles that of the cervix and is physiologically dominated by Lactobacillales, but Clostridiales, Bacteroidales, and Actinomycetales are also regularly detected. Bacterial community differences and shifts are almost exclusively detected between different *Lactobacillus* strains without any negative impact on pregnancy outcomes. During pregnancy, microbial richness and diversity are reduced along with an increased bacterial load. At the same time, the prevalence of potential pathogens like microbial species of ureaplasma and mycoplasma is reduced. The taxonomic composition of the microbial community of the vagina remains stable during pregnancy with an increase of the microbial diversity before birth of the healthy infant at term. These data lead to the conclusion that the composition of the vaginal microbiota is tightly regulated during pregnancy and that the switch to the nonpregnant situation precedes and maybe even triggers birth [9–13].

Microbial diversity and orchestrated structural changes during early life are key features for a healthy microbiome. Ethnicity and regional differences have a key impact on the vaginal microbiota. It remains to be determined whether changes in the vaginal microbiome observed in Hispanic and black women account for the increased rate of preterm delivery in these ethnic groups [14–16].

The fetus swallows huge amounts of amniotic fluid. This explains why its gut gets colonized with the intrauterine microflora already before birth. After birth, the infant gets rapidly colonized by maternal vaginal, gut, and skin microbiota. In the term infant, the mode of delivery either by vaginal birth or by caesarian section determines microbial diversity and whether the gut is primarily colonized by the maternal vaginal and fecal or the skin microbiota. These data are a first scientific indication that early events have a long-term health impact on microbiota structures [17–20]. In contrast to the situation at term, the decision of preterm delivery by caesarian section or vaginal birth does not have an impact on gut microbial diversity and longitudinal microbiota changes and microbiota display a pattern distinct from that at term. As an example of the disparities, preterm microbiota lack *Bacteroides* species, which display a delayed colonization pattern after caesarian section. Although microbiomes are principally able to adapt to that of the term infant within several weeks, the intrauterine and postnatal miscolonization poses a major threat to the health of the preterm infant [21–23]. The following chapters summarize the substantial advances in our understanding of microbiota structures and dynamics during pregnancy and after birth.

3. Pathologic Changes of the Microbiome Associated with Premature Labor and Preterm Delivery

The reasons for preterm labor comprise a multitude of different causes including maternal psychosocial distress, hormonal changes, uterine overdistension, cervical disease, vascular and maternal disorders, and breakdown of the maternal-fetal tolerance [24]. Among these, infection and inflammation are the main drivers of preterm labor and account for at least one half of preterm births [24–26]. Till today, histopathology is the gold standard to determine chorioamnionitis in contrast to the low sensitivity and specificity of clinical evaluation scores and laboratory parameters [27]. In severe chorioamnionitis, a typical pattern with an increase in bacterial abundance and reduced diversity with the dominance of bacteria has been observed, which is not seen in the physiologic situation [28]. The evolutionary attenuation of the maternal and fetal immune system enables the intrauterine growth of the fetus, but immune tolerance of the mother at the same time puts the fetus at risk for infection [29]. The diagnosis of a maternal infection is of crucial importance, as the fetus, exposed to

microbiota in the amniotic cavity, experiences an immunologic adaptation. This new phenomenon is termed the so-called immunotolerance or immunoparalysis. This means that the previous or ongoing exposure to microbiota suppresses the necessary physiologic immune response and that an adequate increase in classical markers of inflammation and infection is not guaranteed [30, 31]. The placental microbiota structures display distinct patterns in term and preterm born infants, independent of the mode of delivery, and can be influenced by living conditions like excessive weight gain during pregnancy [32, 33]. Microbial species of ureaplasma and mycoplasma but also Aerococcaceae, Bifidobacteriaceae, and Fusobacteria predominate or are even exclusively present in the membranes of preterm delivered babies. Conversely, bacterial dysbiosis and inflammation in the fetal membranes can occur without preterm labor and without the typical clinical signs and seems not to put the fetus at risk for preterm delivery [2, 4, 34]. In the situation of preterm labor, a shift of microbiota with reduced microbial diversity takes place. Latest molecular techniques proved superior to detect microbial invasion and diversity compared to conventional culture techniques, which opened a new diagnostic window of opportunity [35]. The long-prevailing concept of bacterial ascension or transmission from the urogenital tract as the main driver of chorioamnionitis and amniotic inflammation was based on the bacteriological detection of microbes typically present. They include bacterial species of the genera *Streptococcus, E. coli, Gardnerella* spp., *Prevotella, Gonorrhea, Treponema, Chlamydia, Ureaplasma, and Mycoplasma* as well as yeasts like *Candida* [13, 36–38]. Further species including anaerobic Fusobacteriaceae were recently detected by the non-cultivation-based techniques.

In fact, the microbiome gets dominated by abundance of bacteria from the urogenital tract, the gut, and the oral cavity (Figure 1). This shift is accompanied by alterations in microbial and metabolic pathways, which are suggested to contribute to preterm labor and birth [34, 39]. The lower urogenital tract and the perianal region constitute a microbial epitope which is highly influenced by the bacterial colonization of the gut and the local viability conditions. Microbiomes change during pregnancy, and differences in composition have long been acknowledged to account for variations in preterm birth rates [11, 16, 40, 41]. The reduction of microbial richness and diversity and changes in microbiome structure seem to occur early, in the first to second trimester of pregnancy, and there seem to exist racial disparities [14, 42–44]. That is, the cervical microbiota from women with *Chlamydia trachomatis* infection, which predisposes for preterm birth, differ completely from that of healthy women with respect to microbial diversity. They display a change in microbial taxa away from *Lactobacillus* species to anaerobes [43, 45]. While *Lactobacillus* species that dominated cervical microbiota have a lower risk of invasion of the amniotic cavity and chorioamnionitis after premature rupture of membranes, the prevalence of *Gardnerella* and *Sneathia* increases the probability [46].

Microbiota from the oral cavity can equally induce chorioamnionitis, when they are translocated via the bloodstream or by sexual practices. Members of the genera *Streptococcus, Porphyromonas, Filifactor, Campylobacter,* and *Fusobacterium* represent species which were repetitively connected with preterm labor. The scientific data point out that gut microbiota constitute the third source for bacterial translocation and can cause amniotic infection and chorioamnionitis. Reduction in microbial diversity and changes in gut microbiota occur during pregnancy with a dominance of Proteobacteria and Acinetobacteria away from *Bifidobacterium*, *Streptococcus*, Clostridiales, and *Bacteroides*, which predisposes for preterm delivery and disease [47–51]. The pathologic changes in the amniotic microbiome are retrieved in meconium samples from preterm infants with a high accordance. Microbiota are shifted to strains of the genera *Enterobacter*, *Enterococcus*, *Photorhabdus,* and *Tannerella*, which are known for their inflammatory, and potentially preterm birth inducing, properties [39, 52, 53]. In line with these observations, the type of nutritional diet or an active inflammatory bowel disease impacts on the gut microbiota composition, and the risk of preterm delivery is increased in these patients. This underlines the concept that a change in maternal gut microbiota is one of the responsible triggers [54].

4. The Consequences for the Premature Infant

Inflammatory diseases represent the biggest threat to the preterm infant and affect all organs, including the immature lung, cardiovascular system, immune system, brain, eye, and gastrointestinal tract, with acute and persistent consequences for the patient's health. Infection accounts for or aggravates acute respiratory distress, leakage, and arterial hypotension after birth. Simultaneously, all organs are at high risk for secondary complications including NEC, nosocomial infection, cerebral damage, retinopathy of prematurity, and endocrinological nonbalance. Infection promotes the establishment of BPD and somatic and developmental disorders [55].

Antibiotic therapy is aimed at combating life-threatening pre- or postnatal infections with pathogenic microbiota, but they cannot reduce or prevent the concomitant inflammatory organ damage. So far, most pathomechanistic insights are available for the inflammatory damage to the immature lung and efficient therapeutic interventions are restricted to a very limited number of drugs. The reason for the so far unsuccessful establishment of effective therapeutic interventions is based on the complexity of the involved pathomechanisms. The complex interplay between different central pathways and persistent cell phenotype distortion after a one-time injury poses further obstacles that need to be bypassed to reach therapeutic efficiency [56–59]. The following sections are dedicated to the detailed description of some of the most important disease burdens provoked by microbial dysbiosis. They summarize the actual status of therapeutic interventions with proven efficacy. Special focus is drawn to highlight the impact of the disturbed endogenous gut microbiome (Figure 2).

5. Microbiota of the Airways and Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) is the chronic lung disease of the preterm infant leading to life-long limitations

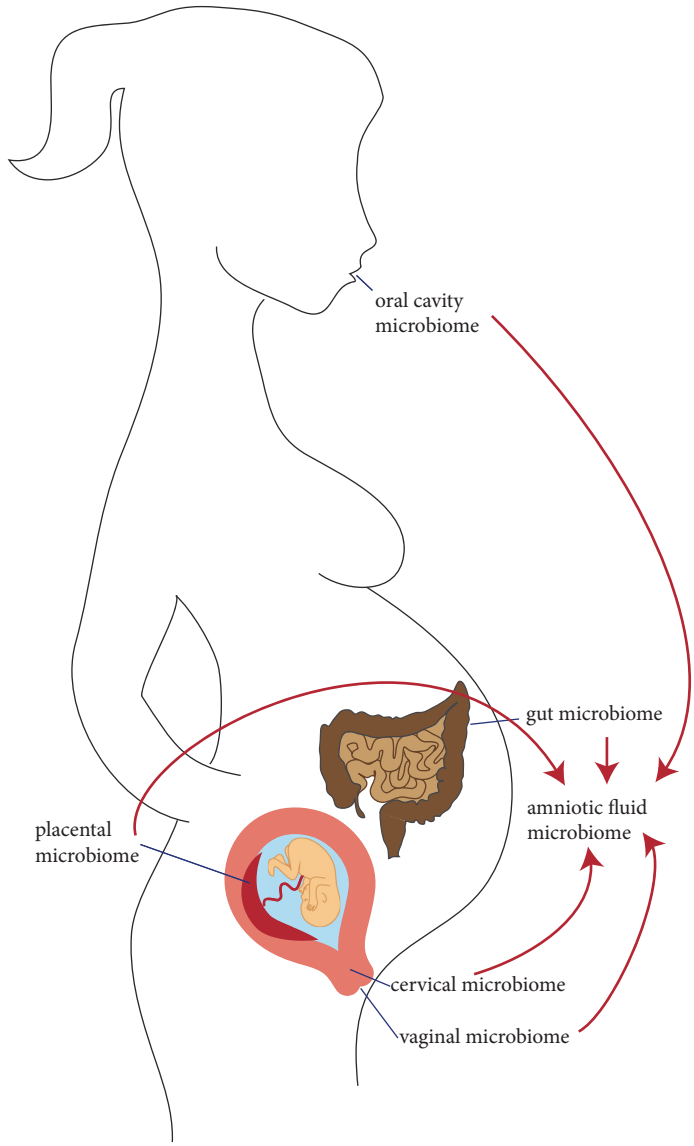

Figure 1: **Origin of microbiota in the amniotic cavity leading to preterm birth:** The microbiome in the amniotic cavity has long been thought to originate exclusively from the vaginal and cervical microbiome. But microbiota from the oral cavity, gut, and even the placenta provide a substantial contribution to the microbiome in the amniotic cavity mainly via haematogenic spread.

in lung function [59]. On a pathophysiologic level, BPD is characterized by distorted alveolar and vascular growth in the saccular stage of lung development. Central to the pathogenesis is the pulmonary inflammatory response after birth, which is mainly provoked by pre- and postnatal infections and the life-saving therapies of mechanical ventilation and oxygen supply [58]. While chorioamnionitis and special pathogens like bacterial species derived from ureaplasma are well acknowledged to contribute to the disease in animal trials and preterm cohort studies, the impact of microbial colonization of the respiratory tract *in utero* and after birth was neglected until recently [60–63]. This is surprising, considering the tremendous impact of the microbiome on other pulmonary diseases and immunity of the lung including asthma, pneumonia, cystic fibrosis, COPD, and even pulmonary fibrosis. The airway of the preterm infant is not sterile at birth and its microbiome is highly influenced by the microbiome of the amniotic fluid. Differences in colonization and clinical parameters allow the categorization into disease clusters, which are predictive for the clinical course and outcome [64]. Reduced microbial diversity at birth, initial abundance of ureaplasma species in tracheal aspirates of ventilated preterm infants, and more pronounced changes in the longitudinal microbial community are associated with higher severity of BPD. The association of a predominance of Proteobacteria and decrease in *Lactobacillus* species in

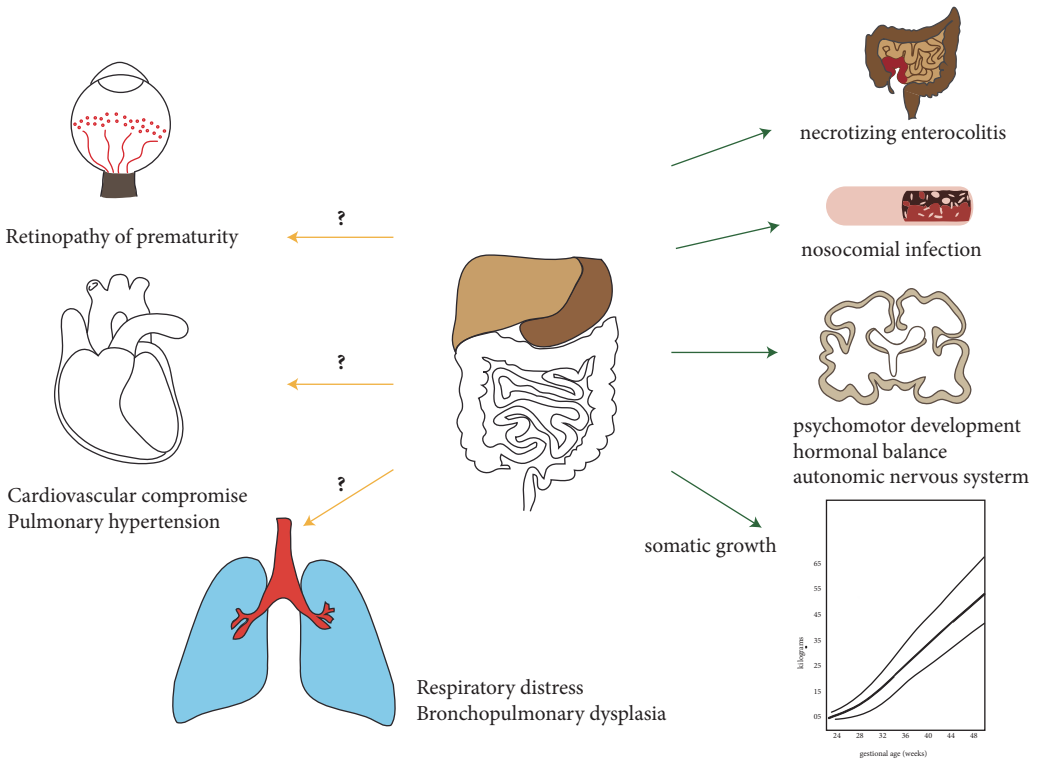

Figure 2: **Impact of gut microbiota on the acute and long-term morbidities in the preterm infant:** The gut microbiota exerts a central influence on human health. In the preterm infant, their impact on NEC and nosocomial infection is well recognized. First studies hint to an important impact on somatic growth, psychomotor development, autonomic regulation, and hormonal balance. In contrast, the contribution to the other acute and long-term sequelae remains to be determined.

the airways of infants with severe BPD was recapitulated in the murine animal model with major impact on the regulation of central lung signaling pathways [65–68]. The well-accepted further dimension arising from the interaction of gut microbiota with the lung in other pulmonary diseases termed the gut-lung axis needs to be established for BPD.

6. The Significant Impact of Microbiota on Necrotizing Enterocolitis

In contrast to BPD, the important contribution of bacteria to the pathogenesis of necrotizing enterocolitis, which constitutes one of the most devastating morbidities of prematurity, is well established. NEC is considered to be a multifactorial disease. The inflammatory response of the gut to microorganisms is a central hypothesis of necrotizing enterocolitis pathogenesis [69, 70]. The great beneficial advantages of breast milk provision are attributed to microbiota diversity and the shaping of immunologic properties [71]. In contrast, antibiotic therapy drives microbial dysbiosis and increases the risk of necrotizing enterocolitis [72]. Derived from these findings, the benefits of prophylactic application of probiotic strains of Bifidobacteria, Lactobacillus, and Saccharomyces was tested in varying experimental and clinical settings. Despite the heterogeneity of results and the need for large-scale meta-analyses, the most recent reviews clearly established a benefit of probiotics to nearly halve the risk of necrotizing enterocolitis and to reduce the incidence of nosocomial infection and death [73, 74].

Postnatally, the gut gets colonized by Gram-positive and Gram-negative bacteria mostly with a facultative or strictly anaerobic metabolism. In the term infant the gut is dominated by Bifidobacteriales and *Bacteroides*. In the preterm infant the presence of a large number of different genera including *Anaerococcus, Aquabacterium, Bacillus, Bifidobacterium, Corynebacterium, Micrococcus, Oceanobacillus, Propionibacterium, Pseudomonas, Rothia, Sarcina, Sneathia,* and *Streptococcus* has been described. As a general phenomenon, the gut microbiome of the preterm infant is dominated by Proteobacteria even when breast milk provision is assured and the appearance of *Clostridium* and *Vellonella* species is retarded [53, 75–78]. The microbiome is highly impacted by pre- and postnatal antibiotic therapy [79]. In this context, probiotic therapy aims to establish and maintain physiologic gut microbiota structures.

A strong dominance of Gram-negative bacteria and a decrease in anaerobic bacteria are described before onset of clinical symptoms of necrotizing enterocolitis, but whether altered microbial structures predispose for necrotizing enterocolitis or are a consequence of gastrointestinal or immunologic immaturity remains an open question. The

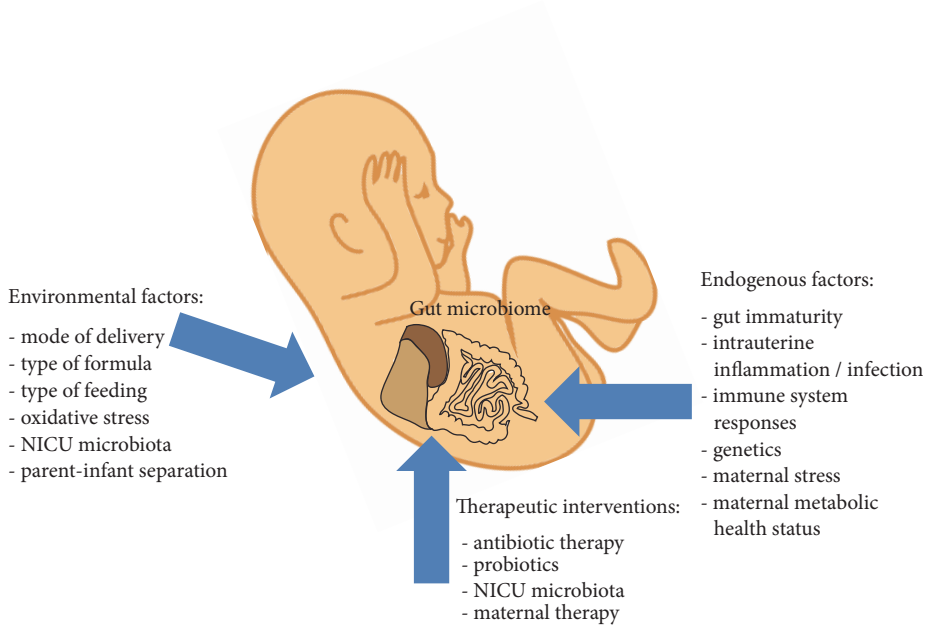

Figure 3: **Factors determining the composition of gut microbiota in the preterm infant:** The microbiome of the gastrointestinal tract of the preterm infant varies widely from that at term and is impacted by a plenty of endogenous and environmental factors and pre- and postnatal therapeutic interventions.

discrepancies in microbiome structures between the feces and samples obtained from the oral cavity or stomach and the impact of microbial dysbiosis of the upper gastrointestinal tract are awaiting further clarification. Presence of Gram-negative bacteria and staphylococci allows the conclusion of their acquisition from the NICU environment [80]. Microbial colonization can be separated into peripartal acquisition as described, i.e., for *Escherichia coli* and *Candida albicans* and hospital acquired microbial structures including *Klebsiella*, *Enterobacter,* and *Acinetobacter* species and *Candida* species other than *Candida albicans* [81]. Taken together, studies of the intestinal microbiome revealed a reduction of bacterial diversity and a shift of microbiota from Bacteroidetes and Firmicutes towards Proteobacteria and potentially pathogenic species including *Staphylococcus aureus, Escherichia coli, Shigella* spp., *Citrobacter* spp., and *Klebsiella* spp. before the onset of clinical symptoms of necrotizing enterocolitis [78, 82–85].

7. Microbial Dysbiosis and the Risk for Nosocomial Infection

Nosocomial infections pose a special risk to the premature infant. Due to the immaturity and immunologic incompetence of the immune system, the preterm infant is particularly vulnerable to nosocomial infections in the hostile environment of the neonatal intensive care unit [30, 31, 86–90]. Furthermore, therapies with antenatal steroids and magnesium as well as small-for-gestational-age status and antenatal smoke exposure seem to further impact and diminish the immunologic response capacity [91–94]. Therefore, skin and gut microbiota colonization and maturation are important prerequisites to prevent pathogen overgrowth and nosocomial infection. The skin of the healthy term and preterm infant is dominated by Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes exposing them to nosocomial infection [95, 96]. The physiologic dominance of gut microbiota structures by Bifidobacteria may serve as a protective factor from gut-epithelial translocation. The differences in gut microbiota between preterm and term born infants at the onset of sepsis are used as an explanation for the vulnerability of the preterm infant and the important role of the gut microbiome in disease initiation. Concordance of microbiota isolated from the gut of infants with sepsis and bacteria identified in positive blood cultures supports this assumption [97–99]. The actual pathomechanistic understanding suggests the following sequence: The preterm infant is exposed to the hostile environment of the neonatal intensive care unit and gut microbiota display a big disparity after birth. A uniform microbiome is established within the first weeks of life with prevalence of highly pathogenic bacteria including *Staphylococcus, Enterococcus,* and several Enterobacteriaceae, while Bifidobacteria are infrequently detected. Reduction in microbial diversity with predominance of Staphylococci is again another feature predisposing for late-onset infection [100]. Not surprisingly, pre- or postnatal antibiotic therapy reduces microbial diversity and impedes the establishment of physiologic gut microbiota with a shift from Firmicutes and Bacteroidetes towards Proteobacteria and Actinobacteria [85, 101–103].

Taken together, the gut microbiome of the preterm infant is highly impacted by endogenous and environmental factors and maternal and postnatal therapeutic interventions which accounts for the high susceptibility for nosocomial infection (Figure 3).

8. The Gut-Brain Axis in Prematurity

Brain development and function undergo fundamental steps in the last trimester and in the first year of life. The physiologic steps of brain folding and the developmental steps of the brain connectome far outreach the increase in brain volume and highly impact functionality. Their functional importance can be derived from preterm infants with severe limitations in brain functions, which display tremendous alterations in these critical steps [104, 105]. More and more data from animal and human studies support the finding that the gut microbiome, especially at early postnatal stages, has tremendous impact on behavioral and stress responses later in life [106–110]. The term gut-brain axis summarizes not only the multiple and complex functions of the cerebrum, but endocrine homeostasis, the sympathetic-parasympathetic, and even the enteric nervous system. The neurologic disorders comprise diseases like autism spectrum disorders, depression, and anxiety which are frequently observed in former preterm infants and restrict their quality of life beyond intelligence and gross and fine motor functions [111, 112]. Even persisting hormonal dysregulations in former preterms are coming into the focus of research [113]. Underlining the functional relevance of the gut-brain axis to the neurodevelopmental outcome after prematurity, higher and persisting prevalence of *Bifidobacteria* in the gut microbiome is associated with improved scores for mental development at 24 months [114]. Convincing animal data demonstrate the far-reaching impact of pre- and postnatal microbiota changes on brain development and the different functional regions which were reproduced in first association studies in children [115–117]. The gut-brain axis is not a one-way but the gut and the brain impact each other bidirectionally which can lead to multiplication in effect size [118–120]. This comes especially true as the preterm infant is exposed to high stress levels and repeated painful procedures [121]. Future studies will have to elucidate how microbiota modulate brain development and function physiologically compared to preterms, how these early life events lead to persisting psychomotor sequelae, and whether microglia cells are the only targets within the central nervous system [122]. *Vice versa*, it remains to be determined how impaired brain function impacts physiologic microbiota structures.

9. Breast Milk and Beyond to Shape Physiologic Microbiota Structures

Breast milk is the optimal nutrition of the preterm infant with respect to acute and long-term health, somatic growth, and psychomotor development. It is well established from studies in healthy newborn that the infant's microbiome is crucially promoted and shaped by the microbiota, anti-inflammatory and antioxidative properties, growth factors, hormonally active substances, and cytokines provided by breast milk feeding. Overall, physiologic gut microbiota establishment and enrichment are facilitated by breast milk [69]. The maternal microbes excreted from the mammary gland, the contact to the skin of the breast, and the nutritional components of breast milk enable the maturation of immune functions and the establishment of a stable physiologic rich and diverse microbiome with a dominance of *Bifidobacteria* and *Lactobacillus* species but also presence of species of the genera *Staphylococcus, Streptococcus, Propionibacterium, Bacteroides, Rothia, Enterococcusi,* and *Pseudomonas* [99, 123–129]. But also strictly anaerobic gut commensals from the Clostridiaceae including *Blautia, Clostridium, Collinsella* and *Veillonella* species were detected in breast milk together with *Coprococcus, Faecalibacterium,* and *Roseburia* species, which were simultaneously isolated from the mothers' breast milk and stool. This allows the conclusion that additionally to the skin-gut axis a maternal gut-breast microbiome axis exists and that the infants' gut microbiome is shaped by the maternal gut microbiome [128, 130, 131]. Gut microbiota composition after preterm birth differs from that of mothers who delivered at term with a shift from *Bifidobacterium* to *Enterococcus* species. It is impacted by perinatal maternal antibiotic therapy with a decrease in *Lactobacillus, Bifidobacterium, Staphylococcus,* and *Eubacterium* species [132]. Formula fed infants display a further reduced microbial diversity and the dominance of Enterobacteriaceae, Coriobacteriaceae, and *Bacteroides* [95, 126]. In preterm infants at high risk of gut microbial dysbiosis, probiotic therapy with the bacterial commensals identified in the previously mentioned studies proved overall efficient to reduce the incidence of necrotizing enterocolitis, sepsis, and death. However, the optimal strain or formula and the duration of application await further exploration [73, 74]. Lactoferrin, a protein of the transferrin family with broad antimicrobial action, stands for the steep rising gain of knowledge about the gut microbiota shaping functions of breast milk. Its recombinant application does not only reduce the risk for device associated infections but modulates the fecal microbiome towards the physiologic situation [133]. In contrast to these medical therapeutic interventions, skin-to-skin care is an easy to apply clinical technique to shape the infants' microbiome. Its consistent provision shapes the oral microbiome of the preterm infant and helps to accelerate its maturation [134].

10. Concluding Remarks

The available data convincingly support the hypothesis that the pre- and postnatal microbiome contributes to premature delivery and to the acute sequelae in the preterm infant. Despite the tremendous scientific progress, we have just scratched the surface to understand the consequences of aberrant microbiota and their dynamic changes to disease initiation and progression. The current data convincingly highlight their impact on nosocomial infection and NEC, which constitute not only a tremendous disease burden to the preterm infant but more importantly entail life-long consequences and considerable lethality. Nonetheless, for most of the acute complications and short-term sequelae including pulmonary and cerebral problems a clear cause-relationship is still missing. It remains to be determined whether pathogenic microbiota also account for the distortion of long-term somatic and psychomotor development in preterm infants which did not suffer from severe acute complications like infection or cerebral hemorrhage.

Therefore, comprehensive and long-term oriented research efforts are urgently needed to cover these important and clinically highly relevant aspects. A comprehensive and mechanistic understanding of the connection between microbial dysbiosis and disease initiation and progression will help to develop new therapeutic concepts aiming to control and restore physiologic microbial structures. Further important topics of research are to gain detailed knowledge on microbial structures and how to avoid of sample contamination and to enable the comparability of studies with respect to techniques and sample preparation [135]. It remains an open question how our lifestyle habits and the genetic background impact the microbiome of the pregnant woman and the frequency of preterm born infants.

The successful implementation of postnatal probiotic therapy and further clinical guidelines raises hopes to reduce the maternal and fetal disease burden in the near future and to come to a targeted or even personalized medicine. Next steps can be derived from the observational studies and should include (1) the design of point-of-care techniques to determine microbial structures onsite and in real time to immediately identify the mother and infant at risk, (2) evaluation of the benefits of personalized medicine strategies of vaginal fluid or feces transplantation to the fetus and newborn, (3) the development of new strategies to detect bacterial infection with high prediction accuracy to avoid unnecessary and prolonged antibiotic therapy and subsequent microbial dysbiosis, and (4) to test more potent alternatives to current classical probiotics including mixtures of different bacterial strains and bacterial metabolites. Each of these areas poses tremendous challenges and opportunities to finally reduce the rates of prematurity and of the associated morbidities. Opening and reaching these new frontiers in perinatal science offer the opportunity to come closer to efficient prevention of preterm birth, which poses an ever greater global burden and challenge.

Disclosure

Birte Staude and Frank Oehmke both share first authorship.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Birte Staude, Frank Oehmke, Tina Lauer, and Harald Ehrhardt drafted the manuscript and designed the figures. Judith Behnke, Wolfgang Göpel, Michael Schloter, Holger Schulz, and Susanne Krauss-Etschmann provided valuable intellectual input and edited and revised the manuscript. All authors read and approved the final version of the manuscript.

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Research Article

Emergency versus Elective Cervical Cerclage: An Audit of Our First Two Years of Service

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One of the biggest obstetric challenges is the diagnosis and management of a short cervix as cervical length has an inverse relationship with risk of preterm birth. A cervical cerclage is a surgical procedure to reduce the risk of preterm birth and can be placed in an elective or emergency setting. This is a retrospective review of cervical cerclages inserted at an outer metropolitan hospital from February 2014 to May 2017. Since the introduction of the service, a total of 43 patients were identified as requiring a cervical cerclage. Four of these patients were transferred to tertiary hospitals. Of the 39 cerclages inserted, 26 were elective and 13 were emergency, placed at a mean gestation of 15.6 and 19.6 weeks. In total, there were 35 live births, 2 stillbirths, and 2 neonatal deaths. The maternal demographics (age, gravidity, parity, and preterm risk factors) were not statistically significant between the two groups. The mean pregnancy prolongation and birthweight was greater in the elective than the emergency group (21.4 versus 14.1 weeks; 3148.2 versus 2447.2 grams). There was no obvious pattern with which patients received antibiotics pre-, intra-, or postoperatively or received a vaginal swab. This audit identified the need for improvements to guidelines to standardise the use of antibiotics and progesterone in women with a cervical cerclage.

1. Introduction

Cervical insufficiency, earlier known as cervical incompetence, is the process of painless cervical dilatation, resulting in second trimester pregnancy loss in an otherwise normal pregnancy [1, 2]. It is thought to occur in 1% of the obstetric population and suspicion is raised when there is a history of recurrent midtrimester loss, previous preterm delivery, or a previous short cervix [2]. One of the biggest obstetric challenges is the diagnosis and management of a short cervix as multiple different definitions and guidelines exist [3].

Cervical cerclage is an obstetric procedure first described in the 1950s, where a suture is placed around the cervix for prevention of preterm birth (PTB) [3]. PTB is defined as delivery prior to 37 weeks of gestations and can lead to increase morbidity and mortality [3–6]. The World Health Organisation defines PTB <28 weeks as extreme preterm, 28-32 weeks as very preterm, and 32-37 weeks as late preterm [7].

The cervical length is most accurately measured using transvaginal ultrasound as it is more reproducible than transabdominal ultrasound [6]. The measurement will vary depending on the gestation, with shortening occurring as the pregnancy continues. At 22-25 weeks of gestation, the median cervical length is expected to be 35mm. The significance of a short cervix is the inverse relationship it has with risk of PTB; the shorter the cervix, the higher the risk of PTB. The risk is increased if other preterm risk factors are present [8].

A short cervix is defined as one which is less than the 10th centile (25mm) in the midtrimester pregnancy [5, 8, 9]. Studies have shown that interventions at this cervical length improve pregnancy outcomes in both low and high-risk women [9]. The management of patients with short cervix can include surveillance, progesterone pessaries, or insertion of a cervical cerclage and there is evidence that such interventions may reduce the risk of PTB [3, 5, 10, 11].

A cervical cerclage is frequently categorised as an elective or emergency cerclage [4, 9]. An elective cerclage is usually placed at the end of the first trimester. The indication for an elective cerclage is based on prior obstetric history. An emergency cerclage can be placed up to 24 weeks of gestation and is indicated when there is a visibly dilated cervix on speculum examination or if there has been an unexpected finding of

Table 1: Maternal demographics.

	All Cerclage (n=39)	Elective Cerclage (n=26)	Emergency Cerclage (n=13)	P-Value (Elective vs Emergency)
Mean maternal age	31.3	31.7	30.6	
Mean gravidity	4.0	4.0	3.7	
Mean parity	1.7	1.7	2.0	
Mean BMI (kg/m^2)	27.3	27.0	27.8	
Risk Factors (%)				
MTL	27 (69.2)	20 (76.9)	7 (53.8)	0.16
PTL	7 (17.9)	7 (26.9)	0 (0)	0.07
Cervical surgery	9 (23.1)	6(23.1)	3 (23.1)	1.0
Uterine	2 (5.1)	2 (7.7)	0 (0)	0.543

MTL: midtrimester loss.
PTL: preterm loss.
Uterine: uterine congenital malformations.

a shortened cervix on routine ultrasound examination [4, 10–12]. Trials looking at outcomes between emergency and elective cerclage have shown that pregnancy outcomes are comparable, although some have also shown poorer obstetric outcomes in the emergency cohort [12, 13].

Sunshine Hospital is an outer Melbourne secondary hospital. A cervical surveillance clinic for the prevention of PTB and midtrimester miscarriage was established in 2014. A guideline for the management of a short cervix in this institution was implemented in 2015. In order to review the outcomes of all cervical cerclages placed during in this period, a retrospective audit of all cerclages placed from 2014 to 2017 was conducted.

2. Method

Data of all singleton pregnant women who underwent an elective or emergency cervical cerclage between February 2014 and May 2017 at a single secondary obstetric centre were reviewed. Ethics approval was provided by the Ethics Committee at Western Health. No funding was received for this study. Patient data were collated from the hospital's electronic systems, Birthing Outcome Systems (BOS) and BOSSNET. The data was collected onto a Microsoft Excel spread sheet. A statistical online package was used for the Pearson Chi-squared test analysis.

In the elective cerclage group, the need for a cerclage was determined by past risk factors. These risk factors included previous PTB below 30 weeks' gestation, midtrimester loss, cervical surgery, cervical trauma, or congenital uterine malformations. In the emergency cerclage group, the need for a cerclage was identified by an unexpected ultrasound finding of a shortened cervix (less than the 10th centile in midtrimester) or clinically from a speculum examination (dilated cervix with or without bulging membranes).

Patient demographics collected included age, gravidity, parity, and body mass index (BMI) as well as any cervical insufficiency risk factors. The indication for cervical cerclage and other factors related to the cerclage insertion were collected including cervical length and/or dilatation, presence of 'sludge', type of cerclage, and suture material used. Delivery outcomes reviewed included gestation at delivery, birthweight, neonatal survival, and the presence of preterm prelabour rupture of membranes (PPROM) or chorioamnionitis. The use of antibiotics in the group of women receiving a cerclage was reviewed to determine if a standardised approach to prescribing existed and if there was adherence to the institutional guideline.

3. Results

All patients underwent a McDonald Cerclage under general anaesthesia with the majority using Mersilene tape as the preferred suture material. All cerclages were placed by a senior registrar (under consultant Obstetrician supervision) or by a consultant Obstetrician.

In total, there were 43 women identified as requiring a cervical cerclage. Three women were transferred to a tertiary centre due to the unavailability of a suitably trained surgeon. Of the 39 cerclages inserted at our service, 26 were elective and 13 were emergency cerclages. In the elective cerclage group, there were 25 live births and 1 stillbirth. In the emergency cerclage group, there were 10 live births, 1 stillbirth, and 2 neonatal deaths (NND) (Figure 1).

Maternal demographics and risk factors are summarised in Table 1. The mean age, gravidity, parity, and BMI was similar in the two groups. The number of risk factors for cervical insufficiency was increased in the elective cerclage group.

The mean gestational age at cerclage insertion was 15+6 weeks in the elective group compared with 19+6 weeks in the emergency group. Women in the elective group had a mean cervical length of 27.2mm with no cervical dilatation whilst women in the emergency group had a mean cervical length of 14.6mm and a mean 16mm dilated cervix. There was a statistically significantly higher rate of 'sludge' present in the emergency cerclage cohort (7.6 versus 61.5% $p<0.00068$). Following placement of an emergency cerclage, there was greater use of antibiotics and vaginal progesterone. (23.1 versus 69.3% $p<0.0126$ and 57.7 versus 92.3% $p<0.033$) (Tables 2 and 3).

Table 2: Cervical results.

	Total Cerclage (n=39)	Elective Cerclage (n=26)	Emergency Cerclage (n=13)	P-Value (Elective vs Emergency)
Mean cervical length (mm)∗	23.0	27.2	14.6	
Mean cervical dilation (mm)+	16.0	0	16.0	
Presence of sludge (%)	10 (25.6)	2 (7.6)	8 (61.5)	0.000688∗∗
Mersilene Suture Material (%)	27 (69.2)	18 (69.2)	9 (69.3)	1.0
Cervical Cultures (%)				
Candida	8 (20.5)	6 (23.1)	2 (15.4)	0.694
Ureaplasma	2 (5.1)	1 (3.8)	1 (7.7)	1.0
BV	1 (2.6)	1 (3.8)	0 (0)	1.0

∗*Total n= 31, Elective n= 21, and Emergency n=10.*
Others excluded as length not specified in report.
+*Total n=6; Emergency n=6.*
Others excluded as dilation not present or not specified in report.
∗∗ *Statistically significant.*

Table 3: Antibiotic and progesterone use.

	Total Cerclage (n=39)	Elective Cerclage (n=26)	Emergency Cerclage (n=13)	P-Value (Elective vs Emergency)
Antibiotic Pre-Op (%)	3 (7.7)	1 (3.9)	2 (15.4)	0.253
Antibiotic Intra-Op (%)	28 (71.8)	18 (69.2)	10 (76.9)	0.719
Antibiotic Post-Op (%)	15 (38.5)	6 (23.1)	9 (69.3)	0.0126∗
Progesterone Pre-Op (%)	12 (20.8)	6 (23.1)	6 (46.2)	0.163
Progesterone Post-op (%)	27 (69.2)	15 (57.7)	12 (92.3)	0.033∗

∗Statistically significant.

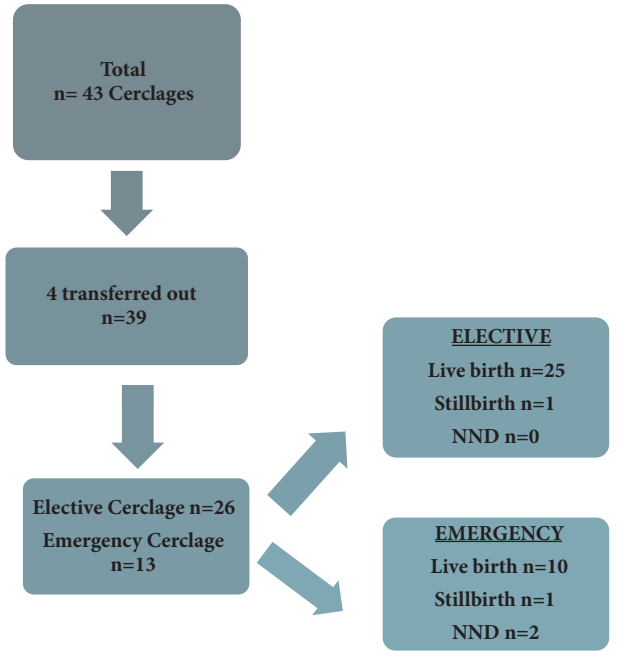

Figure 1: Pregnancy outcomes.

Women were more likely to deliver at a later gestation in the elective group with a mean gestation of 37 weeks compared with 34 weeks in the emergency cerclage group. The mean pregnancy prolongation and birthweights were greater in the elective cerclage group. An increased rate of preterm prelabour rupture of membranes (PPROM) was observed in the emergency cerclage group with 75% occurring in the extreme preterm period. The rate of extreme preterm PPROM was 16.7% in the elective cerclage group. There were no identified cases of chorioamnionitis in either group (Table 4).

4. Discussion

This retrospective review found that elective placement of cervical cerclage had a trend towards better pregnancy outcomes as has been previously established. A greater pregnancy prolongation period was observed with an expected greater mean birthweight compared with the emergency cerclage group. The rates of neonatal death and stillbirths was reduced in the elective cerclage group. Pregnancy prolongation decreases PTB, which improves neonatal morbidity and mortality and also reduces the economic burden on the healthcare system. The recent Western Australia Preterm Birth Prevention Initiative has been working towards such goals [11].

The placement of an emergency cerclage conferred benefits with a mean pregnancy prolongation of 14+1 weeks, improving neonatal survival. The liveborn rate was 76.1% with a mean gestation of 33.7 weeks at delivery. There was

Table 4: Obstetric and pregnancy outcomes.

	Total Cerclage (n=39)	Elective Cerclage (n=26)	Emergency Cerclage (n=13)
Mean GA cerclage placed (weeks)	16.9	15.6	19.6
Mean GA at delivery (weeks)	35.9	37.0	34
Mean Pregnancy Prolongation (weeks)	19.0	21.4	14.1
Mean Birthweight (grams)	2914.6	3148.2	2447.2
Chorioamnionitis (%)	0 (0)	0 (0)	0 (0)
PPROM (%)	10 (24.6)	6 (23.0) *(i) 1(16.7): extreme preterm* *(ii) 1 (16.7): very preterm* *(iii) 4 (66.7): late preterm*	4 (30.8) *(i) 3 (75): extreme preterm* *(ii) 1 (25): late preterm*
Liveborn (%)	35 (94.5)	25 (96.2)	10 (76.2)
NND (%)	2 (5.1)	0 (0)	2 (15.4)
Stillborn (%)	2 (4.1)	1 (3.8)	1 (7.7)

GA: gestational age.
PPROM: premature prelabour rupture of membranes.
NND: neonatal death.

an increased rate of PPROM in the emergency group (23% versus 30.8%) but no other cerclage associated complications. It is known that the risk of complications can be higher in the setting of an emergency cerclage [12].

In the emergency group, the two NND and the one stillbirth were all in women with pregnancies that resulted in PPROM at an extreme preterm gestation. In the elective cerclage group, the only stillbirth was also following PPROM at an extreme preterm gestation. This finding is similar to previous studies which have demonstrated that emergency cerclage has a higher risk of poorer obstetric outcomes [13].

There was also a statistically higher rate of 'sludge' present in the emergency cerclage cohort (7.6 versus 61.5% $p<0.00068$). Sludge is intra-amniotic debris which is in close proximity to the internal cervical os. It is thought to be a marker for subclinical intra-amniotic infection, but this remains contentious [14]. A recent study by Adanir et al. demonstrated that sludge is an independent risk factor for PTB [14].

In this audit, there were significant inconsistencies with the use of antibiotics pre-, intra-, and postoperatively in both type of antibiotic used and length of use in our cohort. There are no trials that support the use of antibiotics with cerclage; however broad-spectrum antibiotics are commonly prescribed intraoperatively [13]. The institutional guideline gave no guidance for the use of antibiotics with cervical cerclage.

The guideline does recommend performing vaginal swabs in the presence of a short cervix and treatment for organisms known to potentially contribute to preterm labour but is not explicit in its recommendation pre-cerclage. Furthermore, there is no evidence to support the routine use of progesterone with cervical cerclage; however in our cohort, progesterone was used frequently after insertion, particularly in the emergency cerclage group [5].

The limitations to this study are the small number of cases identified which can limit the generalisability of this study and the retrospective nature of this review.

5. Conclusion

This audit has identified with the introduction of a cervical surveillance clinic at Sunshine Hospital that women at risk of midtrimester miscarriage and PTB are being identified and management instituted. The outcomes for both elective and emergency cerclage are good with low rates of complications documented. There are inconsistencies with some aspects of care in women receiving cervical cerclages with deficiencies in the institutional guideline available for clinicians. This audit supports the need for a cervical surveillance clinic and an improved evidence based guideline. The will aid in standardising the care for at risk women for better pregnancy outcomes.

Data Availability

This information can be provided on request. The data collected is on a password protected excel file.

Disclosure

An earlier version of this abstract was presented in the manuscript in Abstracts of the 22nd Annual Congress of the Perinatal Society of Australia and New Zealand (PSANZ), 25–28 March 2018, Auckland, New Zealand.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Adiponectin and Omentin Levels as Predictive Biomarkers of Preterm Birth in Patients with Gestational Diabetes Mellitus

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Objective. The aim of this study was to determine any changes in adiponectin and omentin levels in GDM patients who delivered at term and preterm and to evaluate whether adipokines can be useful as a clinical biomarker to predict subsequent preterm delivery. *Patients and Methods*. The levels of adiponectin and omentin were measured in four groups: (1) women with GDM who delivered at term (n=63); (2) women with GDM who had the symptoms of threatened preterm labor and delivered at term (n=23); (3) women with GDM and spontaneous preterm birth (before 37 completed weeks of gestation) (n=19); (4) women with physiological pregnancy (n=55). *Results*. In comparison with control group the median adiponectin concentrations were significantly lower in all GDM groups (10737 versus 8879; 7057; 6253 ng/ml, respectively; $p<0.01$). The median omentin concentrations were also significantly lower in all GDM groups in comparison with control group (469 versus 432; 357; 308 ng/ml, respectively; $p<0.01$). No significant differences in adiponectin and omentin levels between the GDM, preterm labor, and preterm birth groups were observed. However, there was a trend towards lower adiponectin and omentin levels in preterm birth group. The strong correlations between adiponectin and omentin levels were observed in all groups ($R=0.801$, $p<0.001$; $R=0.824$, $p<0.001$; $R=0.705$, $p<0.001$; $R=0.764$, respectively; $p<0.001$). In the univariable logistic regression model, significant correlation between omentin concentrations and preterm birth occurrence was found. *Conclusions*. Our findings suggest that omentin-1, rather than adiponectin, could be useful as a predictor of preterm birth in patients with gestational diabetes mellitus.

1. Introduction

Preterm birth (PTB) is defined as any birth before 37 completed weeks of gestation or fewer than 259 days since the first day of the last menstrual period [1]. The worldwide frequency of PTB is unchanged over two decades. Every year, an estimated 15 million infants are born preterm globally [2]. Decreasing gestational age at delivery is connected with higher infant mortality and disability risk. PTB is an important precursor to future morbidity in both developed and developing countries [3].

PTB is a syndrome with a variety of causes and can be divided into two main groups: iatrogenic preterm birth (30-35%) and spontaneous preterm birth (65-70%). The exact cause of spontaneous preterm labor and delivery can not be determined in almost one-half of all cases [4, 5]. The pathophysiology of PTB also differs between spontaneous and medically indicated births. Main causes of PTB include stress, incompetent cervix, placental ischemia, decidual hemorrhage, placental abruption, systemic or cervical maternal genital tract infections, uteroplacental insufficiency, multiple gestation, and chronic conditions such as high blood

pressure, gestational diabetes, blood clotting disorders, and maternal periodontal disease [6].

Other factors are obesity or underweight before and during pregnancy, having a previous premature birth, in vitro fertilization, extreme maternal age (<17 or >35 years old), smoking cigarettes, nonwhite race, and physical injury or trauma [5].

It has been published that the risk for spontaneous preterm delivery decreases as maternal body mass index (BMI) increases. Obese women also have fewer contractions and longer cervical lengths and are more likely to deliver after their due date [7, 8]. The reason for this protective effect is not known and is understudied.

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first detected during pregnancy. It is the most common metabolic disorder of pregnant women and represents one of the main problems in perinatal medicine. Prevalence of GDM may range from 5 to 20% of all pregnancies, depending on the population tested and the screening methods employed. GDM is associated with adverse maternal and neonatal outcomes. Women with GDM have also significantly elevated risk for type 2 diabetes mellitus (T2DM) in later life [9]. It is agreed that diabetes is associated with preterm birth—either spontaneous or medically indicated. However, some investigators did not notice a higher risk for PTB in patient with GDM [10], but the others reported that GDM by itself is a risk factor for PTB [11]. The results from the HAPO study that shows linear relationship between maternal hyperglycemia and increased risk of birth weight above the 90th percentile, primary cesarean delivery, premature delivery, and shoulder dystocia have been published [12].

Adipose tissue is a highly active endocrine organ and produces a number of adipokines. It has been described that the placenta produces similar adipokines as adipose tissue and that their serum levels can change during the pregnancy. It has been also reported that adipokines might play an important modulatory role in maternal-fetal adaptations and several adipocytokines have been analyzed throughout gestation and their levels have been suggested as biomarkers of pregnancy-related complications including preterm delivery [13, 14].

Adiponectin is a 244-amino-acid-long polypeptide hormone which is a member of adipokines group that modulates a number of metabolic processes. Adiponectin is almost uniquely synthesized by adipocytes but also within the intrauterine environment and stimulates the glucose uptake in skeletal muscles and decreases the hepatic glucose synthesis [15]. However, the signaling pathway of adiponectin (5′AMP-activated protein kinase) has been described as a mechanism important to the myometrium; no data are available about the adiponectin influence on regulation of uterine contractility [15].

Omentin is a novel adipokine of 313 amino acids and is mainly synthesized by visceral adipose tissue. Omentin is also expressed and secreted by the human placenta. Two high isoforms have been described: omentin-1 and omentin-2 with 83% amino acid identity. Omentin-1 is the major circulating form [16]. Omentin levels are inversely correlated with obesity and positively with adiponectin levels [17]. It has been observed that circulating maternal omentin-1 concentrations were higher in normal spontaneous term births than in preterm births. These results suggest that omentin could play an important role in the pathophysiology of preterm delivery [18].

It is possible that an alteration in the maternal endocrine or metabolic status, observed in GDM, and modification in adipokines secretion may have an impact on the functioning of the smooth muscle, particularly uterine contractility, and play the role in preterm delivery [19].

The aim of this study was to determine if there are any changes in adiponectin and omentin levels in GDM patients who delivered at term and preterm and to evaluate whether adipokines can be useful as a clinical biomarker to predict subsequent preterm delivery.

2. Patients and Methods

One hundred and five women with GDM and 55 healthy pregnant women were included in the retrospective study, which was conducted in the Department of Obstetrics and Perinatology at Medical University of Lublin. The blood samples have previously been used to study the gestational diabetes mellitus pathogenesis in normal pregnant women and those with pregnancy complications. All patients signed informed consent to participate in the study, which had previously been approved by the Bioethical Review Board of the Medical University in Lublin. The study was performed according to the principles expressed in the Declaration of Helsinki.

The patients were divided into the following groups: (a) women with GDM who delivered at term (GD group: n=63); (b) women with GDM who had the symptoms of threatened preterm labor and delivered at term (TGD group: n=23); (c) women with GDM and spontaneous preterm birth (before 37 completed weeks of gestation) (PBGD group: n=19); (d) women with physiological pregnancy (PP group: n=55).

Exclusion criteria were as follows: multiple pregnancy, underlying disorders: prepregnancy diabetes mellitus, any form of hypertension, chronic renal disease, liver diseases, inflammation and infectious diseases, systemic lupus erythematosus, antiphospholipid syndrome, and intrauterine growth retardation.

The oral glucose tolerance test (OGTT) with 75 g of glucose according to WHO standards was performed in all women participating in the study and in the control group between 24th and 28th week of gestation. GDM was diagnosed on the basis of the following WHO criteria: fasting ≥92 mg/dL (5.1 mmol/L), at 1st hour ≥180 mg/dL (10.0 mmol/L), and at 2nd hour ≥153 mg/dL (8.5 mmol/L) [20].

Information about current and previous pregnancies, maternal and family anamnesis, maternal age, smoking status, and delivery outcomes was obtained using medical records.

Maternal prepregnancy body mass index (BMI) was calculated from maternal recall of weight prior to pregnancy and the height measured during the first visit before 8th

Table 1: Characteristics of study population (mean and standard deviation).

	GDM (GD group) n=63	GDM and threatened preterm labor (TGD group) n=23	GDM and preterm birth (PBGD group) n=19	Uncomplicated pregnancy (PP group) n=55	p
Maternal age (years)	28.6 (5.1)	27.5 (4.0)	29.1 (4.0)	27.0 (4.5)	NS
Gravidity	2.12 (0.9)	2.38 (1.1)	2.0 (0.85)	2.0 (0.96)	NS
Gestational age at delivery (weeks)	39.3 (0.83)	39.4 (0.9)	32.3 (2.5)	39.4 (0.75)	p<0.001 PBGD vs GD; TGD; PP
Baby's birth weight (g)	3545 (406)	3465 (421)	1876 (161)	3410 (374)	p<0.001 PBGD vs GD; TGD; PP
Pre-pregnancy BMI (kg/m^2)	24.5 (2.6)	23.8 (2.4)	24.8 (2.5)	22,5 (1.8)	p<0.05 PBGD vs PP
BMI at sampling (kg/m^2)	26.8 (2.2)	26.4 (1.6)	26.7 (1.98)	25.0 (1.5)	NS
BMI at delivery (kg/m^2)	28.8 (2.3)	28.7 (1.4)	28.1 (2.0)	27.2 (1.3)	p<0,05 GD vs PP
Estimated fetal weight at sampling (g)	980 (206)	898 (174)	875 (161)	981 (152)	NS
Gestational age at sampling (weeks)	27.0 (1.5)	26.3 (1.3)	26.1 (1.2)	26.9 (1.3)	NS

week of gestation. BMI was recalculated at the time of blood sampling and at the time of delivery.

The blood samples for research tests were taken together with the samples for routinely performed laboratory tests. Serum levels of adiponectin and omentin were obtained at 24–28 weeks of gestation. The samples were allowed to clot for at least 30 minutes before centrifugation at 1000 G, which was continued for 20 minutes. Serum has been removed and then frozen at −70°C. The adiponectin concentration was measured by enzyme-linked immunosorbent assay technique (Human Adiponectin Elisa, High Sensitivity, BioVendor R&D Products, Czech Republic) as well as the omentin-1 concentration (Human Omentin-1 Elisa, BioVendor R&D Products, Czech Republic), according to the manufacturer's instructions.

3. Statistical Analysis

The obtained data were assessed by the one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test (Statistica, v. 12, StatSoft, Inc., Tulsa, OK, USA). Data distribution was assessed using Shapiro-Wilk test. Results with normal distribution are presented as the means ± standard deviation (SD); data with skewed distribution are presented as the medians with minimum and maximum values (Min./Max). To assess the correlation of the data, Pearson's and Spearman's correlation tests were performed. Herein, $p < 0.05$ was considered as a statistically significant difference. Univariable logistic regression models were used for calculations odds ratios (ORs) with 95% confidence intervals (CIs) predicting spontaneous preterm birth based on adiponectin and omentin concentrations.

4. Results

The studied groups did not differ with regard to the baseline descriptors: age, gravidity, estimated fetal weight, gestational age, and BMI at sampling. Expected differences in neonatal birth weight and gestational age at delivery were observed between PBGD and the other groups (Table 1). Prepregnancy BMI was higher in preterm birth group than the controls (24.8±2.5 versus 22.5±1.8 kg/m^2, $p<0.05$) (Table 1).

In comparison with control group the median adiponectin concentrations were significantly lower in all GDM groups (10737 versus 8879; 7057; 6253 ng/ml, respectively; $p<0.01$). No significant differences in adiponectin levels between the GDM, preterm labor, and preterm birth groups were observed. However, there was a trend towards lower adiponectin level in preterm birth group (Table 2).

The median omentin concentrations were also significantly lower in all GDM groups in comparison with control group (469 versus 432; 357; 308 ng/ml, respectively; $p<0.01$) and no statistically significant differences between the GDM, preterm labor, and preterm birth groups were observed. As in the case of adiponectin, there was also a trend towards lower omentin level in preterm birth group (Table 2).

The strong correlations between adiponectin and omentin levels were observed in GD, TGD, PBGD, and PP groups ($R=0.801$, $p<0.001$; $R=0.824$, $p<0.001$; $R=0.705$, $p<0.001$; $R=0.764$, respectively; $p<0.001$) (Table 3).

Pairwise correlations between adiponectin and omentin-1 levels and clinical and demographic characteristics (maternal age; gravidity; prepregnancy, at sampling, and at delivery BMI; gestational age and estimated fetal weight at sampling;

Table 2: Median (range) concentrations of adiponectin and omentin in blood sampling.

	GDM (GD group) n=63	GDM and threatened preterm labor (TGD group) n=23	GDM and preterm birth (PBGD group) n=19	Uncomplicated pregnancy (PP group) n=55	p
Adiponectin ng/ml	8879 (3832-16348)	7057 (3429-12907)	6253 (3607-10801)	10737 (5294-18842)	p<0.01 PP vs GD; TGD; PBGD
Omentin ng/ml	432 (195-687)	357 (258-588)	308 (244-519)	469 (307-799)	p<0.01 PP vs GD; TGD; PBGD

Table 3: Correlation between adiponectin and omentin, maternal age, gravidity, gestational age at delivery, baby's birthweight, and BMI.

	GDM		GDM and threatened preterm labor		GDM and preterm birth		Uncomplicated pregnancy	
	Adiponectin		Adiponectin		Adiponectin		Adiponectin	
	R	p	R	p	R	P	R	p
Omentin	0.801	p<0.001	0.824	p<0.001	0.705	p<0.001	0.764	p<0.001
Maternal age (years)	- 0.365	p<0.01	-0.257	NS	-0.483	p<0.05	-0.187	NS
Gravidity	-0.352	p< 0.01	-0.039	NS	-0.003	NS	-0.093	NS
Gestational age at delivery (weeks)	-0.173	NS	0.022	NS	-0.211	NS	-0.024	NS
Baby birthweight (g)	-0.138	NS	-0.058	NS	-0.458	p<0.05	0.083	NS
Pre-pregnancy BMI (kg/m^2)	-0.828	p<0.001	-0.868	p<0.001	-0.814	p<0.001	-0.630	p<0.01
BMI at sampling (kg/m^2)	-0.799	p<0.001	-0.792	p<0.001	-0.703	p<0.01	-0.539	p<0.01
BMI at delivery (kg/m^2)	-0.822	p<0.001	-0.758	p<0.001	-0.738	p<0.001	-0.447	p<0.01
Gestational age at sampling	-0.053	NS	0.082	NS	-0.476	p<0.05	0.011	NS
Estimated fetal weight at sampling	-0.003	NS	-0.049	NS	-0.458	p<0.05	0.018	NS

GDM: gestational diabetes mellitus.
BMI: body mass index.
R: Spearman's correlation coefficient.
p: statistical significance.
NS: statistically not significant.

gestational age at delivery; baby's birth weight) were determined for all groups.

Adiponectin concentration was inversely correlated with prepregnancy, at sampling, and at delivery BMI in GD group (R=-0.828, -0.799, -0.822, respectively), TGD group (R=-0.868, -0.792, -0.758, respectively), PBGD group (R=-0.814, -0.703, -0.738, respectively), and PP group (R=-0.630, -0.539, -0.822, respectively) (Table 3).

As in the case of adiponectin, omentin levels were also inversely correlated with prepregnancy, at sampling, and at delivery BMI in GD group (R=-0.743, -0.712, -0.750, respectively), TGD group (R=-0.809, -0.553, -0.559, respectively),

Table 4: Correlation between omentin and adiponectin, maternal age, gravidity, gestational age at delivery, baby's birthweight, and BMI.

	GDM		GDM and threatened preterm labor		GDM and preterm birth		Uncomplicated pregnancy	
	Omentin		Omentin		Omentin		Omentin	
	R	p	R	p	R	P	R	p
Adiponectin	0.801	p<0.001	0.824	p<0.001	0.705	p<0.001	0.764	p<0.001
Maternal age (years)	-0.358	p<0.05	-0.029	NS	-0.336	NS	-0.187	NS
Gravidity	-0.290	p<0.05	-0,091	NS	-0.016	NS	-0.091	NS
Gestational age at delivery (weeks)	-0.267	p<0.05	-0.111	NS	-0.150	NS	-0.075	NS
Baby birthweight (g)	0.001	NS	-0.061	NS	-0.152	NS	-0.048	NS
Pre-pregnancy BMI (kg/m^2)	-0.743	p<0.001	-0.809	p<0.001	-0.800	p<0.001	-0.604	p<0.01
BMI at sampling (kg/m^2)	-0.712	p<0.001	-0.553	p<0.01	-0.760	p<0.001	-0.509	p<0.01
BMI at delivery (kg/m^2)	-0.750	p<0.001	-0.557	p<0.01	-0.756	p<0.001	-0.430	p<0.01
Gestational age at sampling	-0.067	NS	-0.013	NS	-0.310	NS	-0.048	NS
Estimated fetal weight at sampling	0.001	NS	-0.061	NS	-0.200	NS	-0.030	NS

GDM: gestational diabetes mellitus.
BMI: body mass index.
R: Spearman's correlation coefficient.
p: statistical significance.
NS: statistically not significant.

PBGD group (R=-0.800, -0.760, -0.756, respectively), and PP group (R=-0.04, -0.509, -0.430, respectively) (Table 4).

There was a correlation in GD group between prepregnancy BMI and gestational week at delivery (R=0.24, p<0.05), baby's birthweight (R=0.262, p<0.050), and maternal age (R=0.472, p< 0.001), but no correlations were found between BMI at sampling and at delivery and these parameters.

In the PBGD group the correlation between prepregnancy, at sampling, and at delivery BMI and maternal age was found (R=0.667, p<0.01; R=0.663, p<0.01; R=0.724, respectively; p<0.001).

In the univariable logistic regression model, no significant correlation between adiponectin and preterm birth was found.

We have found the correlation between the omentin concentration and the risk of preterm delivery. When we compared PBGD group versus PP group, omentin levels were inversely correlated with the risk of preterm birth (ß =-0.0188, p=0.0006, OR = 0.9814, 95% CI: 0.9710 - 0.9919). An increase of omentin level by 100 ng/ml decreases the possibility of preterm birth by almost 75%. When we compared PBGD group versus GDM group, omentin levels were also inversely correlated with the risk of preterm birth (ß =-0.0084, p= 0.017, OR= 0.9916, 95% CI: 0.9848 - 0.9985) and an increase of omentin level by 100 ng/ml decreases the possibility of preterm birth by almost 57%.

5. Discussion

In our study we have found the lower adiponectin levels in all GDM groups in comparison with uncomplicated pregnancy group, but we have not found significant differences in adiponectin levels between the women with GDM and GDM preterm labor and GDM preterm birth group. However, we noticed a trend towards lower adiponectin level in preterm birth group. In the univariable logistic regression model, no significant correlation between adiponectin and preterm birth was found.

Catalano et al. have noticed that the maternal adiponectin secretion progressively declines during the pregnancy. On the other hand, it has been published that the adiponectin levels during 1st, 2nd, and 3rd trimester were similar and were significantly higher than in postpartum period. Authors were of the opinion that despite the increased insulin resistance on the course of pregnancy, there were no significant changes in the adiponectin concentrations. This may signify that the regulation of the adiponectin release during gestation is altered [21]. It has not been confirmed whether there is adiponectin secretion from the placenta. Adiponectin can be found in fetal circulation after 24th week of gestation and the level tends to increase as pregnancy progresses. The fetal adiponectin levels have been observed to be significantly higher than those in the mother [22].

Adiponectin is adipokine, which in relation to GDM was the most widely studied. The data analyzing the adipokines levels in physiological and complicated pregnancies are ambiguous. It has been published that level of adiponectin was significantly lower in first and early second trimester in pregnant patient who later had diagnosed GDM [23]. Cortelazzi et al. have noticed that the levels of circulating adiponectin were lower in women with GDM as compared to patient in uncomplicated pregnancy [24]. A correlation between significantly decreased concentrations of adiponectin and beta cell impaired function in pregnant patient has been found, suggesting it may be an early marker of GDM development [25]. Mohammadi et al. analyzed the relationship of adiponectin levels to GDM and glucose intolerance. They measured the concentrations of serum adiponectin in GDM and healthy pregnant patients, who were screened between 24 and 28 weeks of pregnancy and compared the concentrations between the groups. Serum adiponectin concentrations were significantly lower in patients with GDM (5.10±2.15 ng/mL versus 7.86±3.52 ng/mL, $p = 0.001$) than in healthy pregnant women. These outcomes showed that serum concentrations of adiponectin were significantly lower in gestational diabetic women [26]. Doruk et al. investigated adiponectin levels in women with GDM and normal glucose tolerance (NGT) at 24-28 gestational weeks. Fasting serum adiponectin, glucose, and glycated hemoglobin (HbA1c) were determined in 88 pregnant women, 44 with GDM and 44 with NGT. Serum adiponectin levels were significantly reduced in GDM compared with the NGT group. The GDM group delivered significantly earlier than the NGT group. Adiponectin serum concentration was significantly reduced in patients with adverse outcomes and cesarean sections [27].

Although the signaling pathway of adiponectin (AMPK) has been described as a mechanism important to the myometrium, no data about the adiponectin effect on uterine contractility are available. A very limited number of articles focused on the adiponectin and preterm birth have been published. Mazaki-Tovi et al. examined pregnant women, who were divided into four groups: (1) uncomplicated pregnancy; (2) with an episode of preterm labor and intact membranes without intra-amniotic infection/inflammation (IAI) who delivered at term; (3) preterm labor without IAI who delivered preterm; and (4) preterm labor with IAI who delivered preterm. They analyzed levels of serum adiponectin multimers total, high-molecular-weight (HMW), medium-molecular-weight (MMW), and low-molecular-weight (LMW).) Lower median maternal serum concentration of total and HMW adiponectin was associated with preterm labor leading to preterm delivery or with an episode of preterm labor which does not lead to preterm delivery. Patients with preterm labor and IAI had the lowest median concentration of total and HMW adiponectin as well as the lowest median HMW/total adiponectin ratio. These findings confirm our results [28].

Vieira et al. in their multicentre study analyzed the factors, which could be predictors of uncomplicated pregnancy at birth. They examined obese (BMI$\geq$ 30 kg/m^2) pregnant women at 15^{+0} to 18^{+6} weeks of gestation to find sociodemographic, clinical, and biochemical factors. They identified them using multivariable logistic regression. These factors are multiparity, lower maternal age, systolic blood pressure, HbA1c levels, and elevated adiponectin levels (OR= 1.4(1.18-1.66); CI=95%) [29].

Kominarek et al. compared adipokines between experiencing preterm labor (PLT) and prior preterm deliveries (PTD). They analyzed serum levels of leptin, adiponectin, and resistin during pregnancy (three times: 23-34 weeks, 35-36 weeks, and at delivery). There were no significant differences in adipokines serum levels in patients with PLT and PTD, but they found correlation between body mass index (BMI) and levels of leptin and adiponectin. Researchers also compared levels of adipokines between patients who delivered at term and preterm. No significant differences were found, but levels of adiponectin were higher in preterm delivery group between 23 and 34 weeks and at delivery and lower between 35 and 36 weeks than in term delivery group [30].

Chervenak et al. tested mid-trimester amniotic fluid from 571 pregnant women for adiponectin, interleukin- (IL-) 6, IL-8, and α-amylase to identify adiponectin's associations with maternal parameters, pregnancy outcomes, and mediators in amniotic fluid. Adiponectin median levels were elevated in women with preterm premature rupture of membranes (pPROM) and women with an iatrogenic preterm birth (IPTB) in comparison to mothers with a term delivery ($p= 0.0003$). Higher median levels of adiponectin were also measured in patients whose infants developed fetal growth restriction and in patients whose babies had respiratory distress syndrome ($p<0.0001$). They also observed that adiponectin concentration was positively correlated with amylase level and inversely correlated with maternal body mass index. One of the limitations of study was ethnicity—most of the patients were Asian (309) [31].

In our study we have found the lower omentin levels in all GDM groups in comparison with uncomplicated pregnancy group, but we have not found significant differences in omentin levels between the women with GDM and GDM preterm labor and GDM preterm birth groups. However, we noticed a trend towards lower adiponectin level in preterm birth group. We have found the statistically significant inverse

correlation between the omentin concentration and the risk of preterm delivery.

Omentin is a adipocytokine with insulin sensitizing effects in adipose tissues, but its function in vasculature is not clearly understood. Omentin has been described to participate in diverse pathophysiological processes. Yamawaki et al. have described that omentin inhibited TNF-α-induced cyclooxygenase-2 (COX-2) expression via activation of adenosine 5′-monophosphate-activated protein kinase (AMPK), which further activated the endothelial nitric oxide synthase (eNOS)/NO pathway [32]. Moreover, omentin induces a nitric oxide (NO)-mediated endothelium-dependent vasorelaxation, suggesting that omentin can take a part in the regulation of blood pressure through directly modulating contractile reactivity of blood vessels. However, the specific receptor for omentin has not been isolated yet [33]. The mechanisms by which the omentin can play a role in preterm birth are currently not very well understood. Nitric oxide relaxes also myometrial smooth muscle cells. Thus, we can speculate that similar relaxation effect of omentin could be found in the uterus. In preterm deliveries abnormal activation of proinflammatory cytokines was also described [34]. In *in vitro* studies Qi et al. have revealed that omentin-1 through the inhibition of COX-2 can also act as an anti-inflammatory agent [35]. Moreno-Navarrete et al. have noticed that omentin serum concentrations were inversely correlated with IL-6, TNF-α, and CRP levels and that can confirm the anti-inflammatory effects of this adipokine [36].

Omentin-1 is the major circulating form of omentin and very limited data on its concentrations in gestational diabetes mellitus are available. Similar omentin concentrations between GDM patients and pregnant controls were described and, on the other hand, Barker et al. demonstrated significantly decreased maternal circulating omentin concentrations in GDM compared with healthy pregnant patients. They have also found association of maternal obesity in pregnancy with decreased circulating omentin levels. Prospective studies of omentin as a predictor of gestational diabetes have not been published [37].

Several publications describing omentin in association with pregnancy have been published [37–39]. However, we have found only one article analyzing omentin-1 in relation to preterm birth. Šplíchal et al. compared levels of maternal omentin-1 and genetic variability between spontaneous preterm and term births. They evaluated omentin-1 levels and role of the omentin-1 Val109Asp (rs2274907) polymorphism in 30 pregnant patients with spontaneous preterm birth (sPTB) (16 with preterm premature rupture of membranes (pPROM) and 14 without pPROM) and 32 women with spontaneous term birth (sTB). Levels of maternal omentin-1 were significantly decreased in patients with sPTB, but there were no differences between women with and without pPROM. Scientists also observed no significant effect of the omentin-1 Val109Asp polymorphism on serum levels of omentin-1 [18]. They also revealed, in the univariate model, that the omentin-1 concentrations were negatively associated with sPTB occurrence (ß = -0.0029, OR = 0.9972, 95% CI: 0.9946 – 0.9998, p = 0.032) and calculate that if omentin-1 concentrations increase by 100 ng/ml the chance of sPTB occurrence decreases by approximately 25%. We have also found the same correlation between the omentin concentration and the risk of preterm delivery. Their findings confirm our omentin-1 results and what is also important, the examined population was also Caucasian.

Omentin-1 is mainly expressed in visceral adipose tissue. It has been described that omentin levels were negatively associated with the amount of visceral adipose tissue and BMI. We have found that omentin levels were inversely correlated with prepregnancy, at sampling, and at delivery BMI in all groups. In opposite to our results Šplíchal et al. did not find significant correlations between maternal omentin-1 levels and preconception BMI or BMI at delivery either in term births or in preterm births [18].

Barker et al. revealed that maternal omentin-1 levels were inversely correlated with fetal birth weight and fetal ponderal index [37]. In our study we did not observe any significant correlations between omentin concentrations and baby's birthweight.

The strong correlations between adiponectin and omentin serum levels were observed in all analyzed groups. To the best of our knowledge, this is the first study describing this association in pregnancy.

In presented study, in all analyzed groups, the negative correlation between adiponectin and omentin levels, and the prepregnancy, at sampling, and at delivery BMI were observed. Our observations partially confirm the results of the study presented by MacLachlan et al. who have found that the adiponectin levels are strongly related to BMI in pregnant women at 24-29th weeks of gestation [40]. Other report has revealed no correlation between the adiponectin concentrations and BMI in pregnant women [41]. Brandt et al. have found significant negative association between third trimester circulating maternal omentin levels and several maternal metabolic indices in a normal glucose tolerance population, including maternal BMI, insulin levels, and insulin resistance [42].

6. Conclusions

In conclusion, the adiponectin and omentin concentrations are decreased in all patients with gestational diabetes mellitus. The strong correlations between adiponectin and omentin levels were observed in all groups.

Our study suggests that circulating maternal adipokines may play a role in the pathophysiology of preterm birth. No significant differences in adiponectin and omentin levels between the GDM, preterm labor, and preterm birth groups were observed. However, there was a trend towards lower adiponectin and omentin levels in preterm birth group.

We have found the correlation between the omentin concentration and the risk of preterm delivery. Our findings suggest that omentin, rather than adiponectin, could be useful as a predictor of preterm birth in patients with gestational diabetes mellitus. On the other hand we know that further studies are required to confirm our outcomes. We

hope that these results can help to identify pregnant patients, who need special care during pregnancy.

The limitations of the study also have to be acknowledged. All patients were Caucasian so the findings might not be applicable to other populations. The number of patients were relatively small. Thus, the analysis of outcomes may be underpowered. A larger scale research is recommended to address the limitations. However, we still continue our study, but in our opinion the findings were very interesting to show them on this stage of the study.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

DREAM Is Involved in the Genesis of Inflammation-Induced Prolabour Mediators in Human Myometrial and Amnion Cells

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Preterm birth is the primary cause of perinatal morbidity and mortality worldwide. Inflammation induces a cascade of events leading to preterm birth by activating nuclear factor-κB (NF-κB). In nongestational tissues, downstream regulatory element antagonist modulator (DREAM) regulates NF-κB activity. Our aims were to analyse DREAM expression in myometrium and fetal membranes obtained at term and preterm and to determine the effect of DREAM inhibition on prolabour mediators in primary myometrial and amnion cells. DREAM mRNA expression was significantly higher in fetal membranes obtained after spontaneous labour compared to nonlabour and in amnion from women with histological preterm chorioamnionitis when compared to amnion from women without chorioamnionitis. In primary myometrial and amnion cells, the effect of DREAM silencing by siRNA was a significant decrease in the expression of proinflammatory cytokine IL-6, the chemokines IL-8 and MCP-1, the adhesion molecule ICAM-1, MMP-9 mRNA expression and activity, and NF-κB transcriptional activity when stimulated with the proinflammatory cytokine IL-1β, the bacterial products fsl-1 or flagellin, or the viral dsRNA analogue poly(I:C). These data suggest that, in states of heightened inflammation, DREAM mRNA expression is increased and that, in myometrial and amnion cells, DREAM regulates proinflammatory and prolabour mediators which may be mediated via NF-κB.

1. Introduction

Preterm birth, defined as delivery prior to 37 weeks of gestation, affects approximately 15 million pregnancies annually and is the primary cause of perinatal morbidity and mortality worldwide [1]. Spontaneous preterm birth accounts for up to 70% of all cases [2]. Globally, more than one million preterm babies die each year, and those who survive have significantly higher rates of health complications, such as respiratory distress, jaundice, cerebral palsy, and cognitive impairments, compared to those born at term [3, 4]. Intensive short- and long-term care for these babies poses a significant economic challenge, while the emotional toll borne by their families, also, is immense [5]. Despite clinical interventions and extensive research, preterm birth rates continue to rise. A more thorough understanding of the mechanisms of human parturition is essential to designing new and effective strategies for the prevention and management of preterm labour.

Healthy term labour is widely acknowledged to be a physiological, inflammatory state characterised by leukocytic infiltration of the myometrium, cervix, and fetal membranes [6, 7]. The subsequent release of proinflammatory cytokines, such as interleukin- (IL-) 1β, facilitates the processes of parturition, with elevated levels found in myometrium, amnion, cervical tissue, and amniotic fluid in association with labour [7, 8]. IL-1β can amplify production of cytokines and chemokines, including IL-8 and monocyte chemoattractant protein-1 (MCP-1), and upregulate the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), which promotes further leukocyte recruitment [9]. They also induce cyclooxygenase-2 (COX-2) expression, resulting in uterotonic prostaglandin production [10, 11], and that of matrix metalloproteinase- (MMP-) 9, an extracellular

matrix remodelling enzyme implicated in cervical ripening and membrane rupture [12]. Spontaneous preterm birth is thought to result from untimely and pathological activation of this pathway due to infection, haemorrhage, uterine distention, obesity, or stress, among others [2, 13]. These disease processes share the feature of amplified local or systemic inflammation. Of these, infection has the greatest clinical significance [14] and initiates the aforementioned cascade of inflammatory events via toll-like receptor (TLR) activation by bacterial or viral products.

The nuclear factor-kappa B (NF-κB) signalling pathway is classically associated with inflammation and, in gestational tissues, is a critical regulator of prolabour mediators [15, 16]. NF-κB is highly inducible by IL-1β and microbial products, while NF-κB recognition elements are found within genes encoding IL-1β, IL-6, IL-8, and TNF-α, creating a positive feedback loop. Expression of the RelA subunit is significantly increased in myometrium and amnion in association with labour [17, 18]. Furthermore, NF-κB inhibition has been shown to dampen expression of prolabour mediators in response to proinflammatory stimuli in myometrium, amnion, and placenta [19–21] and also delay time to delivery in mice [22]. Thus, controllable modulation of NF-κB signalling may be of value in preventing spontaneous preterm birth.

Downstream regulatory element antagonist modulator (DREAM), also known as calsenilin and KChIP3, belongs to the neuronal calcium sensor family and has recently been shown to play a role in NF-κB signalling [23–25]. It primarily exists in the cytosol, but nuclear and plasma membrane localisation have also been reported [26, 27]. Three isoforms are known to exist, with molecular weights of 29.1 kD, 26.7 kD, and 26.3 kD. Initial identification of DREAM encompassed various biological processes, including senile plaque production in Alzheimer's disease, pain sensation, membrane excitability, and synaptic plasticity [27–29]. DREAM-deficient mice consistently display attenuated responses to inflammatory pain models and have decreased levels of NF-κB-transcribed proinflammatory mediators in animal models of inflammatory lung and vascular injury [24, 25, 30]. Additionally, DREAM has been shown to bind to promoters of anti-inflammatory cytokines, suppressing their transcription [31]. Only one study has investigated the role of DREAM in pregnancy tissues. DREAM mRNA expression is upregulated in placentas from women with severe early onset preeclampsia [32], another adverse pregnancy outcome associated with inflammation.

Given the central role of inflammation and NF-κB signalling in the processes of human labour and delivery, it was hypothesised that (i) human labour and infection would be associated with increased DREAM expression in human myometrium and fetal membranes and (ii) DREAM silencing would be associated with decreased expression and release of prolabour mediators in the presence of proinflammatory stimuli. Thus, the aims of this study were to (i) characterise the expression of DREAM in human myometrium and fetal membranes obtained from labouring and nonlabouring women at term and preterm with and without evidence of infection and (ii) determine the effect of DREAM silencing on prolabour mediators in human primary myometrial and amnion cells. The proinflammatory cytokine IL-1β, the TLR2/6 ligand and bacterial product fibroblast-stimulating lipopeptide- (fsl-) 1, the TLR5 ligand and bacterial product flagellin, and the TLR3 ligand and viral dsRNA analogue polyinosinic:polycytidylic acid (poly(I:C)) were chosen as they have been shown to promote the expression of proinflammatory and prolabour mediators in human gestational tissues [21, 33, 34].

2. Materials and Methods

2.1. Ethics Statement. This study was approved by the Research Ethics Committee of Mercy Hospital for Women. Written, informed consent was obtained from all participating women.

2.2. Tissue Collection. Myometrium and fetal membranes were collected for two separate studies: expression studies and cell culture studies. All tissues were obtained from women who delivered healthy, singleton infants. Exclusion criteria were BMI > 30, abnormal antenatal glucose tolerance test results, any underlying medical conditions (for example, diabetes mellitus, macrovascular complications, polycystic ovarian syndrome, and preeclampsia), multiple pregnancies, and presence of fetal chromosomal abnormalities. Tissues were processed in the laboratory within 15 min of delivery.

2.2.1. Tissue Collection for Expression Studies. The full clinical characteristics of the patients used for the expression studies are described elsewhere [35].

Myometrium was obtained from women at term (37–41 weeks' gestation) undergoing (i) elective Caesarean section in the absence of labour and (ii) emergency Caesarean section during active labour ($n = 8$ patients per group). Indications for Caesarean section in the absence of labour were breech presentation and/or previous Caesarean section. Indications for Caesarean section in the presence of labour were placenta praevia, fetal distress, and failure to progress. Myometrial biopsies were obtained from the upper margin of the lower uterine segment incision during Caesarean section. No patients underwent induction or augmentation of labour. Tissue samples were snap frozen in liquid nitrogen and immediately stored at −80°C.

Fetal membranes were obtained from women (i) at term undergoing elective Caesarean section in the absence of labour and (ii) at term after spontaneous labour, spontaneous membrane rupture, and normal vaginal delivery ($n = 9$ patients per group). Indications for Caesarean section were breech presentation and/or previous Caesarean section. No patients underwent induction or augmentation of labour. Tissue samples were snap frozen in liquid nitrogen and immediately stored at −80°C.

Fetal membranes were also obtained from women at preterm birth for two separate studies on preterm labour and preterm chorioamnionitis. For the preterm labour study, fetal membranes (amnion and choriodecidua) were obtained from women (i) undergoing Caesarean section in the absence of labour with intact membranes and (ii) after spontaneous

labour and normal vaginal delivery ($n = 9$ patients per group). For the chorioamnionitis study, only amnion was collected as the choriodecidual tissue was degraded. Amnion was collected from women (i) undergoing Caesarean section in the absence of labour and (ii) undergoing Caesarean section in the absence of labour with histologically confirmed acute chorioamnionitis ($n = 8$ patients per group). Indications for preterm delivery (in the absence of labour) were placenta praevia, placental abruption, antepartum haemorrhage, or rhesus isoimmunisation. All preterm placentas were subject to histopathological examination and fetal membranes were swabbed for microbiological culture studies. Chorioamnionitis was diagnosed pathologically according to standard criteria [36].

2.2.2. Tissue Collection for Cell Culture Studies. For the cell culture studies, fresh amnion and myometrium were obtained from women who delivered healthy, singleton infants at term (37–40 weeks' gestation) undergoing elective Caesarean section in the absence of labour. Primary amnion and myometrial cells were isolated and cultured as previously described [37, 38].

2.3. DREAM siRNA Transfection in Primary Myometrial and Amnion Cells. Primary myometrial and amnion cells were transfected with siRNA. Myometrial and amnion cells at approximately 50% confluence were transfected using Lipofectamine 3000 according to manufacturer's guidelines (Life Technologies; Mulgrave, Victoria, Australia). DREAM siRNA (siDREAM) and negative control (siCONT) were obtained from Ambion (Thermo Fisher Scientific; Scoresby, VIC, Australia). Myometrial cells were transfected with 50 nM siDREAM or 50 nM siCONT in DMEM/F-12 for 48 h followed by treatment with or without 100 pg/ml IL-1β, 250 ng/ml fsl-1, 1 μg/ml flagellin, or 5 μg/ml poly(I:C) for 24 h. Amnion cells were transfected with 50 nM siDREAM or 50 nM siCONT in DMEM/F-12 for 48 h followed by treatment with or without 100 pg/ml IL-1β for 24 h. After the final incubation, cells and media were collected and stored at −80°C until assayed as detailed below. Cell viability was assessed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) proliferation assay as we have previously described [39]. The data is presented as fold change in expression relative to the expression level in the IL-1β, flagellin-, and fsl-1- or poly(I:C)-stimulated siCONT-transfected cells, which was set at 1. Each experiment was performed on amnion and myometrium obtained from six patients.

2.4. NF-κB RelA Luciferase Activity. Possible interactions between DREAM and NF-κB were determined using a luciferase assay, as previously described [40]. Primary myometrial cells, prepared as above, at ~70% confluence were transfected with 0.75 ng NF-κB RelA reporter construct (Qiagen) using FuGENE HD transfection reagent (Promega, Alexandria, NSW, Australia). After 6 h, cells were transfected with 50 nM siDREAM or siCONT (as detailed above) for 48 h. The medium was then replaced with DMEM/F12 containing 0.5% BSA with or without 100 pg/ml IL-1β, 250 ng/ml fsl-1, 1 μg/ml flagellin, or 5 μg/ml poly(I:C), and the cells were incubated at 37°C for an additional 24 h. Cells were harvested in lysis buffer and luminescence activity was measured using a luciferase reporter assay kit (Life Research, Scoresby, Victoria, Australia) and Renilla luciferase flash assay kit (Thermo Fisher Scientific, Scoresby, Victoria, Australia), as per the manufacturer's instructions. The ratio of firefly luciferase level to Renilla luciferase level was determined and results are expressed as a ratio of normalised luciferase activity. The experiments were performed on myometrium obtained from six patients.

2.5. RNA Extraction and qRT-PCR. RNA extraction and qRT-PCR were performed as previously described [40]. Total RNA was extracted from tissues and cells using TRIsure reagent, as per the manufacturer's instructions (Bioline, Alexandria, NSW, Australia). RNA concentration and purity were measured using a NanoDrop ND1000 Spectrophotometer. RNA quality was determined via the $A_{260} : A_{280}$ ratio. RNA was converted to cDNA using the high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific; Scoresby, Vic, Australia) according to the manufacturer's instructions. The RT-PCR was performed using the CFX384 Real-Time PCR detection system (Bio-Rad Laboratories; Gladesville, NSW, Australia) using 100 nM of predesigned and validated QuantiTect primers (primer sequences not available) (Qiagen; Chadstone Centre, Vic, Australia). Average gene Ct values were normalised to the average YWHAZ and succinate dehydrogenase (SDHA) Ct values of the same cDNA sample. Fold differences were determined using the comparative Ct method.

2.6. Enzyme Immunoassays. IL-6 and IL-8 release were assessed using the CytoSet™ sandwich ELISA, as instructed (Life Technologies). MCP-1 and ICAM-1 release were assessed by sandwich ELISA from R&D Systems (Minneapolis, MN, USA), as instructed. The interassay and intra-assay coefficients of variation for all assays were less than 10%.

2.7. Gelatin Zymography. MMP-9 activity was assessed by gelatin zymography on conditioned media collected from primary amnion cells, as previously described [34]. Briefly, proteolytic activity was visualised as clear zones of lysis on a blue background of undigested gelatin. Gels were scanned and inverted using the ChemiDoc XRS system (Bio-Rad Laboratories), and densitometry was performed using the Quantity One Image analysis software (Bio-Rad Laboratories).

2.8. Statistical Analysis. Statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA). For two sample comparisons, an unpaired Student's t-test was used to assess statistical significance between normally distributed data; otherwise, the nonparametric Mann–Whitney U was used. For all other comparisons, the homogeneity of data was assessed by Bartlett's test, and, when significant, data were logarithmically transformed before analysis by a repeated measures one-way ANOVA (with LSD post hoc testing to discriminate among the means). Statistical significance

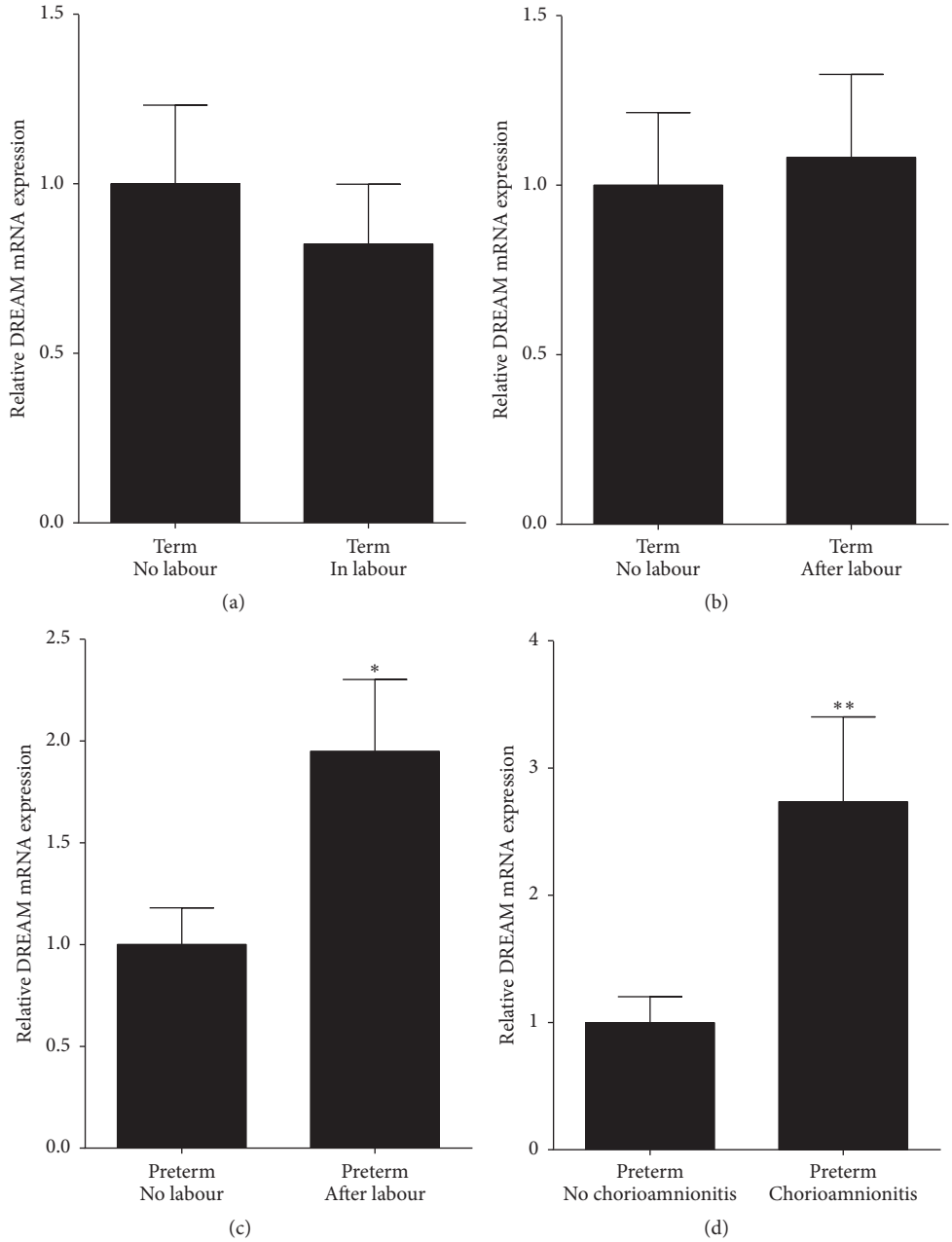

Figure 1: *DREAM expression in human myometrium and fetal membranes*. (a) Human myometrium was obtained from nonlabouring and labouring women at term Caesarean section ($n = 8$ patients per group). (b) Fetal membranes were obtained from women not in labour at term Caesarean section and women after term spontaneous labour onset and delivery ($n = 9$ patients per group). (c) Fetal membranes were obtained from women not in labour at preterm Caesarean section and women after preterm spontaneous labour onset and delivery ($n = 9$ patients per group). (d) Amnion was obtained from women at preterm Caesarean section with or without histological chorioamnionitis ($n = 8$ patients per group). DREAM mRNA expression was analysed by qRT-PCR. All data are displayed as mean ± SEM. $^{*}p \leq 0.05$ versus preterm no labour (Student's t-test); $^{**}p \leq 0.05$ versus preterm no chorioamnionitis (Student's t-test).

was ascribed to a p value ≤ 0.05. Data is expressed as mean ± SEM.

3. Results

3.1. Effect of Term and Preterm Labour and Infection on DREAM Expression in Human Myometrium and Fetal Membranes. We first characterised the expression of DREAM in myometrium and fetal membranes from nonlabouring and labouring women. DREAM mRNA expression was not different in myometrium and fetal membranes obtained from labouring and nonlabouring women at term (Figures 1(a) and 1(b)). On the other hand, in fetal membranes obtained from women at preterm, DREAM mRNA expression was significantly higher in the labouring group compared to the nonlabouring group (Figure 1(c)). Furthermore, at preterm,

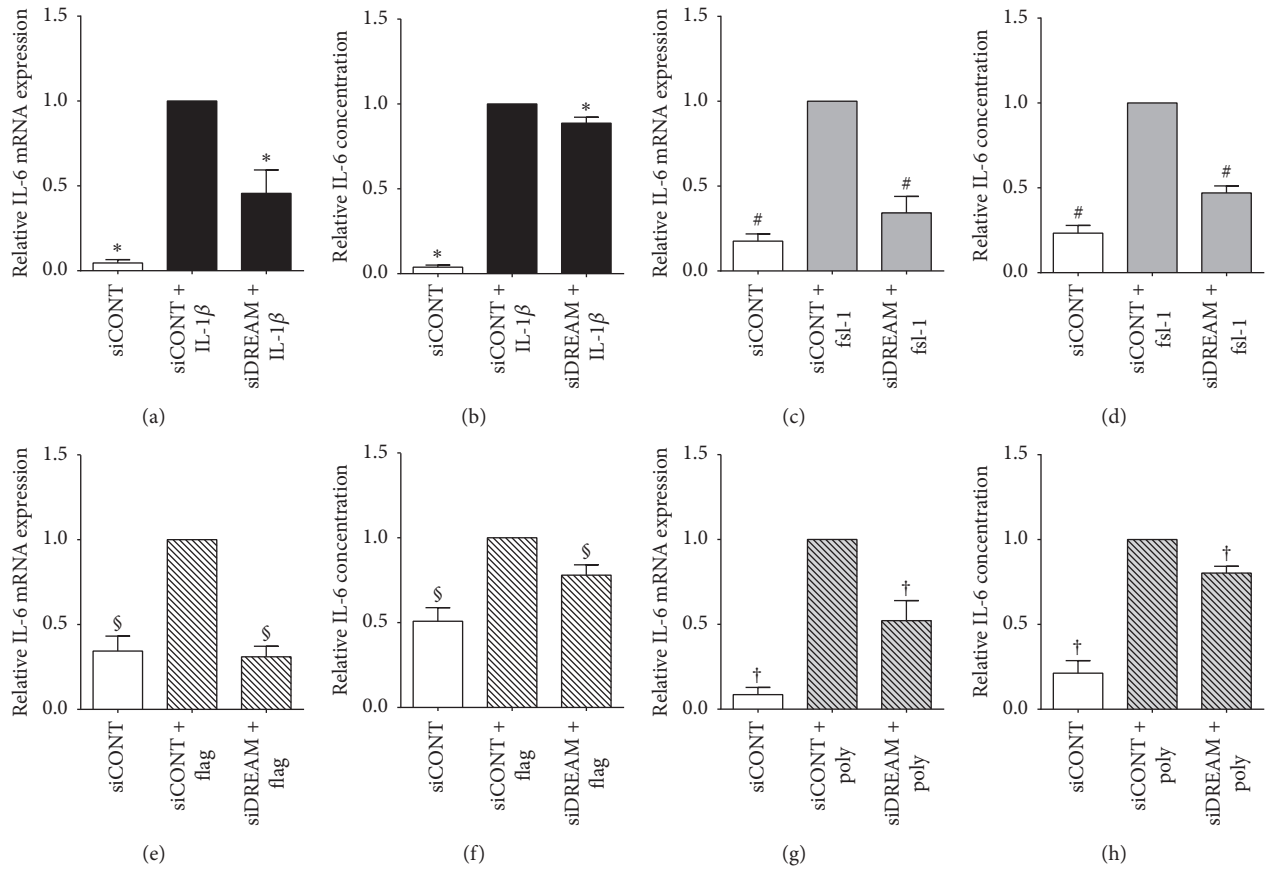

Figure 2: *Effect of siDREAM on the proinflammatory cytokine IL-6 in primary myometrial cells.* Primary myometrial cells were transfected with 50 nM siCONT or 50 nM siDREAM for 48 h and then treated with (a, b) 100 pg/mL IL-1β, (c, d) 250 ng/ml fsl-1, (e, f) 1 μg/ml flagellin, or (g, h) 5 μg/ml poly(I:C) for an additional 24 h (patients). (a, c, e, g) IL-6 mRNA expression was analysed by qRT-PCR. (b, d, f, h) The incubation media was assayed for concentration of IL-6 by ELISA. For all data, fold change was calculated relative to IL-1β-, fsl-1, and flagellin- or poly(I:C)-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^{*}p \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells; $^{\#}p \leq 0.05$ versus fsl-1-stimulated siCONT-transfected cells; $^{\S}p \leq 0.05$ versus flagellin-stimulated siCONT-transfected cells; $^{\dagger}p \leq 0.05$ versus poly(I:C)-stimulated siCONT-transfected cells (one-way ANOVA).

DREAM mRNA expression was also significantly higher in amnion obtained from women with histologically confirmed chorioamnionitis compared to those without histologically confirmed chorioamnionitis (Figure 1(d)). Several attempts to quantify DREAM protein with commercially available antibodies were unsuccessful.

3.2. Effect of siDREAM on Proinflammatory Cytokines, Chemokines, and Adhesion Molecules in Primary Myometrial and Amnion Cells. Functional siRNA studies were performed to determine the role of DREAM in the regulation of prolabour mediators. For these studies, we used primary cells isolated from human myometrium or amnion and treated them with the proinflammatory cytokine IL-1β, the bacterial products fsl-1 or flagellin, and the viral dsRNA analogue poly(I:C) to induce inflammation associated with preterm labour. Following siRNA transfection, primary myometrial cells were treated with IL-1β, fsl-1, flagellin, and poly(I:C). Amnion cells were treated with IL-1β only. Efficacy of transfection was assessed by qRT-PCR. As compared to siCONT-transfected myometrial and amnion cells, siDREAM transfection resulted in a decrease in DREAM mRNA expression by approximately 75%. A MTT cell viability assay showed no difference in absorbance between siCONT- and siDREAM-transfected myometrial (0.63 ± 0.34 versus 0.66 ± 0.27) and amnion (1.49 ± 0.15 versus 1.45 ± 0.15) cells.

The effect of siDREAM on proinflammatory cytokines, chemokines, and adhesion molecules is depicted in Figures 2–4 for myometrial cells and Figure 5 for amnion cells. In siCONT-transfected myometrial cells, treatment with IL-1β, fsl-1, flagellin, and poly(I:C) significantly increased expression of IL-6 (Figure 2), IL-8, and MCP-1 (Figure 3). In siDREAM transfected cells, there was a significant attenuation of IL-6, IL-8, and MCP-1 mRNA expression and secretion when stimulated with all treatments. There was also a significant decrease in fsl-1, flagellin, and poly(I:C)-induced ICAM-1 mRNA expression and secretion in siDREAM transfected cells (Figure 4). There was a significant decrease in IL-1β-induced ICAM-1 mRNA expression, but no effect on secretion of sICAM-1 in siDREAM transfected cells (Figure 4). Similar results were obtained in amnion cells, where siDREAM transfected cells displayed an attenuation

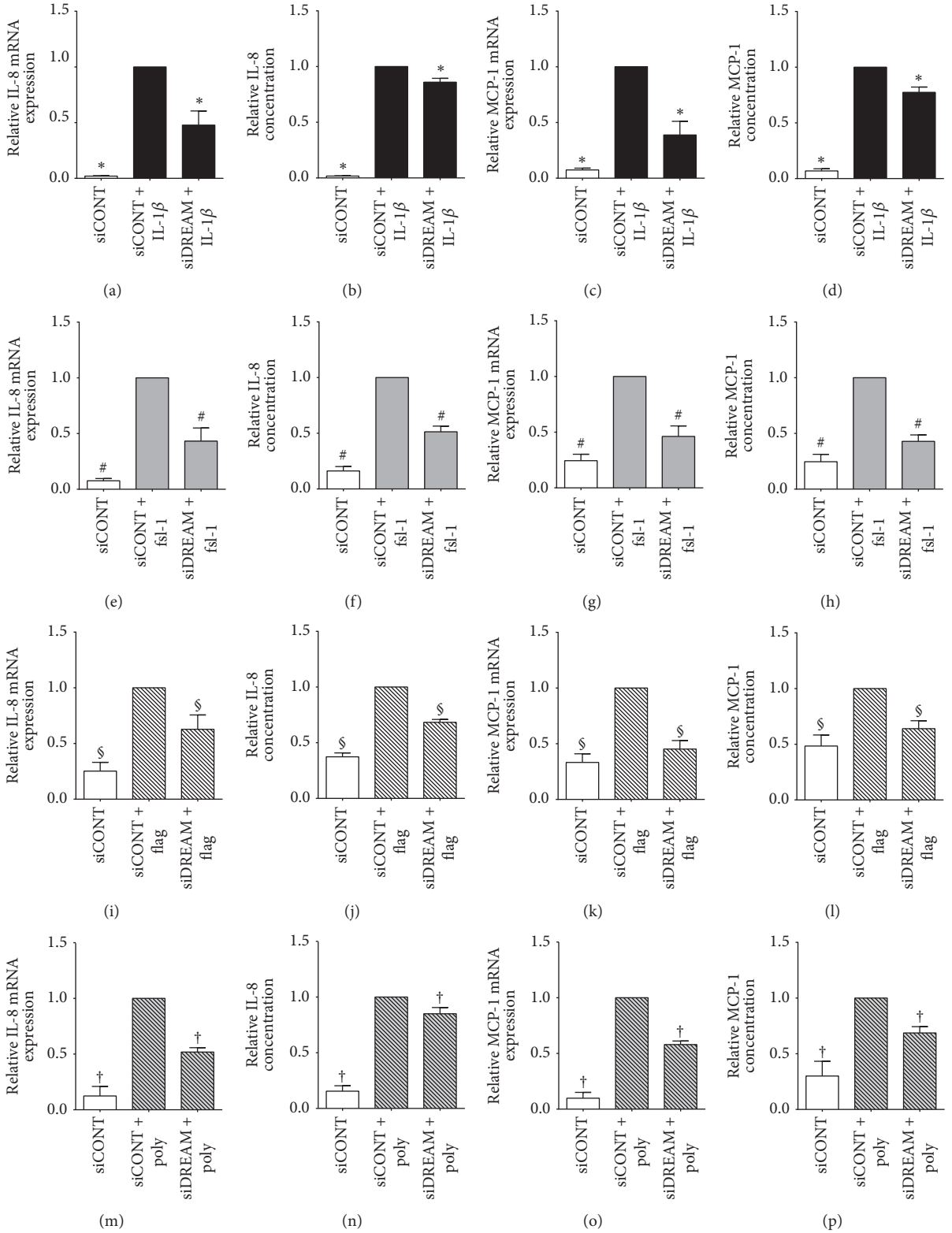

Figure 3: *Effect of siDREAM on chemokines in primary myometrial cells*. Primary myometrial cells were transfected with 50 nM siCONT or 50 nM siDREAM for 48 h and then treated with (a–d) 100 pg/mL IL-1β, (e–h) 250 ng/ml fsl-1, (i–l) 1 μg/ml flagellin, or (m–p) 5 μg/ml poly(I:C) for an additional 24 h ($n = 6$ patients). (a, c, e, g, i, k, m, o) IL-8 and MCP-1 mRNA expression were analysed by qRT-PCR. (b, d, f, h, j, l, n, p) The incubation media was assayed for concentration of IL-8 and MCP-1 by ELISA. For all data, fold change was calculated relative to IL-1β-, fsl-1, and flagellin- or poly(I:C)-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^{*}p \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells; $^{\#}p \leq 0.05$ versus fsl-1-stimulated siCONT-transfected cells; $^{\S}p \leq 0.05$ versus flagellin-stimulated siCONT-transfected cells; $^{\dagger}p \leq 0.05$ versus poly(I:C)-stimulated siCONT-transfected cells (one-way ANOVA).

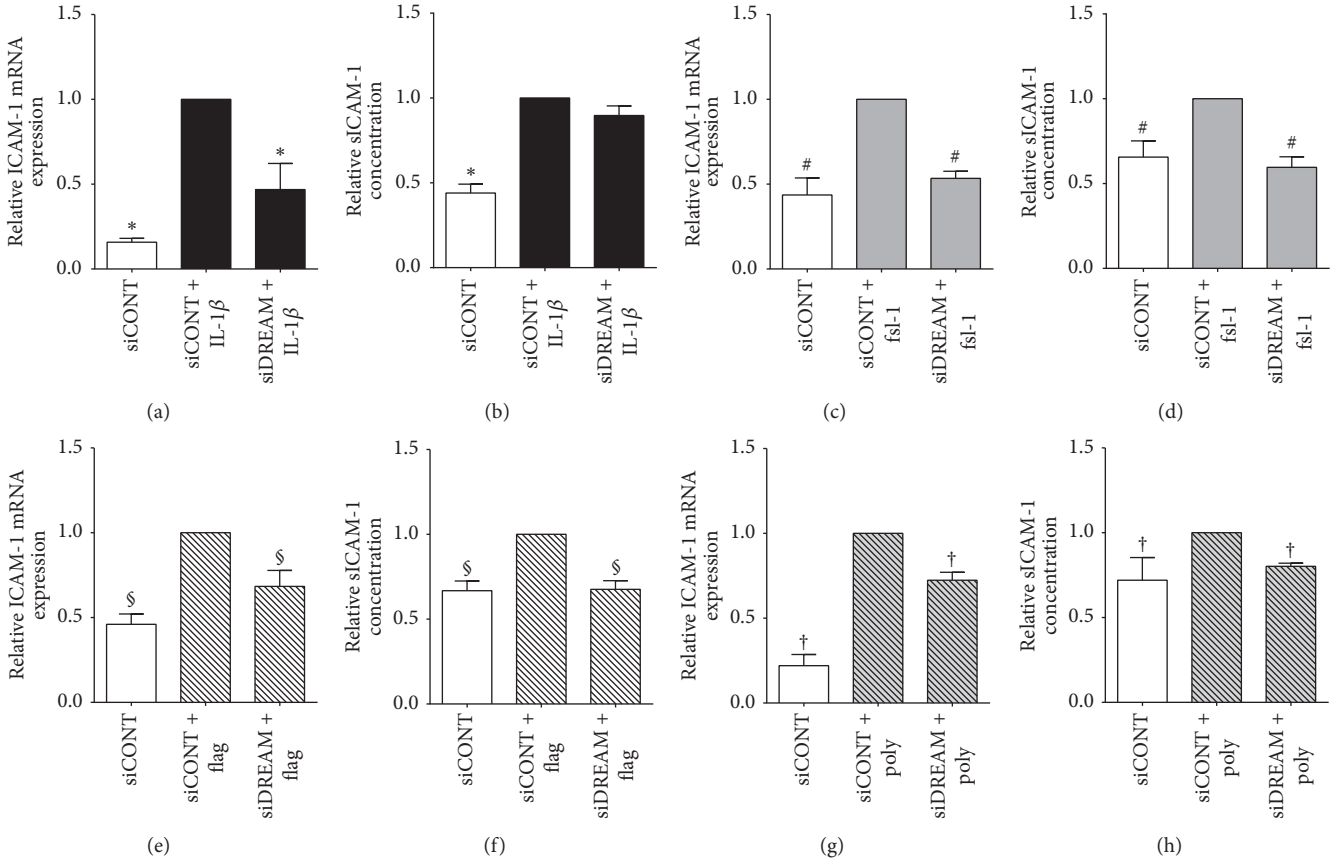

Figure 4: *Effect of siDREAM on the adhesion molecule ICAM-1 in primary myometrial cells*. Primary myometrial cells were transfected with 50 nM siCONT or 50 nM siDREAM for 48 h and then treated with (a, b) 100 pg/mL IL-1β, (c, d) 250 ng/ml fsl-1, (e, f) 1 μg/ml flagellin, or (g, h) 5 μg/ml poly(I:C) for an additional 24 h (n = 6 patients). (a, c, e, g) ICAM-1 mRNA expression was analysed by qRT-PCR. (b, d, f, h) The incubation media was assayed for concentration of ICAM-1 by ELISA. For all data, fold change was calculated relative to IL-1β-, fsl-1, and flagellin- or poly(I:C)-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^{*}p \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells; $^{\#}p \leq 0.05$ versus fsl-1-stimulated siCONT-transfected cells; $^{\S}p \leq 0.05$ versus flagellin-stimulated siCONT-transfected cells; $^{\dagger}p \leq 0.05$ versus poly(I:C)-stimulated siCONT-transfected cells (one-way ANOVA).

of IL-1β-induced IL-6, IL-8, MCP-1, and ICAM-1 mRNA expression and secretion (Figure 5).

3.3. Effect of siDREAM on MMP-9 in Primary Amnion Cells. We also assessed the effect of siDREAM on the expression of the ECM degrading enzyme MMP-9 in primary amnion cells. As expected, IL-1β increased MMP-9 mRNA expression and secretory pro-MMP-9 levels in siCONT-transfected amnion cells (Figure 6). The effect of siDREAM was a significant suppression of IL-1β-induced MMP-9 mRNA expression and pro-MMP-9 production.

3.4. Effect of siDREAM on NF-κB RelA Transcriptional Activity. Finally, we determined if the effects of siDREAM on prolabour mediators may be elicited through NF-κB; Figure 7 demonstrates the effect of siDREAM on NF-κB RelA transcriptional activity. In siCONT-transfected cells, NF-κB RelA transcriptional activity was significantly augmented by IL-1β, fsl-1, flagellin, and poly(I:C) treatment. A significant reduction in IL-1β-, fsl-1-, and flagellin- and poly(I:C)-induced NF-κB RelA transcriptional activity was observed in siDREAM transfected cells.

4. Discussion

A greater understanding of the mechanisms of human parturition is necessary to design new and effective strategies for the prevention of preterm labour. Here, DREAM is identified as a novel therapeutic target. This study is the first to investigate expression and function of DREAM in human myometrium and fetal membranes. While DREAM expression is not altered by labour at term, its expression is significantly increased in fetal membranes after preterm labour and in amnion with histological preterm chorioamnionitis when compared to amnion without histological preterm chorioamnionitis. Functional studies in primary myometrial and amnion cells revealed that DREAM is involved in the production of proinflammatory and prolabour mediators induced by IL-1β, fsl-1, flagellin, and poly(I:C). Additionally,

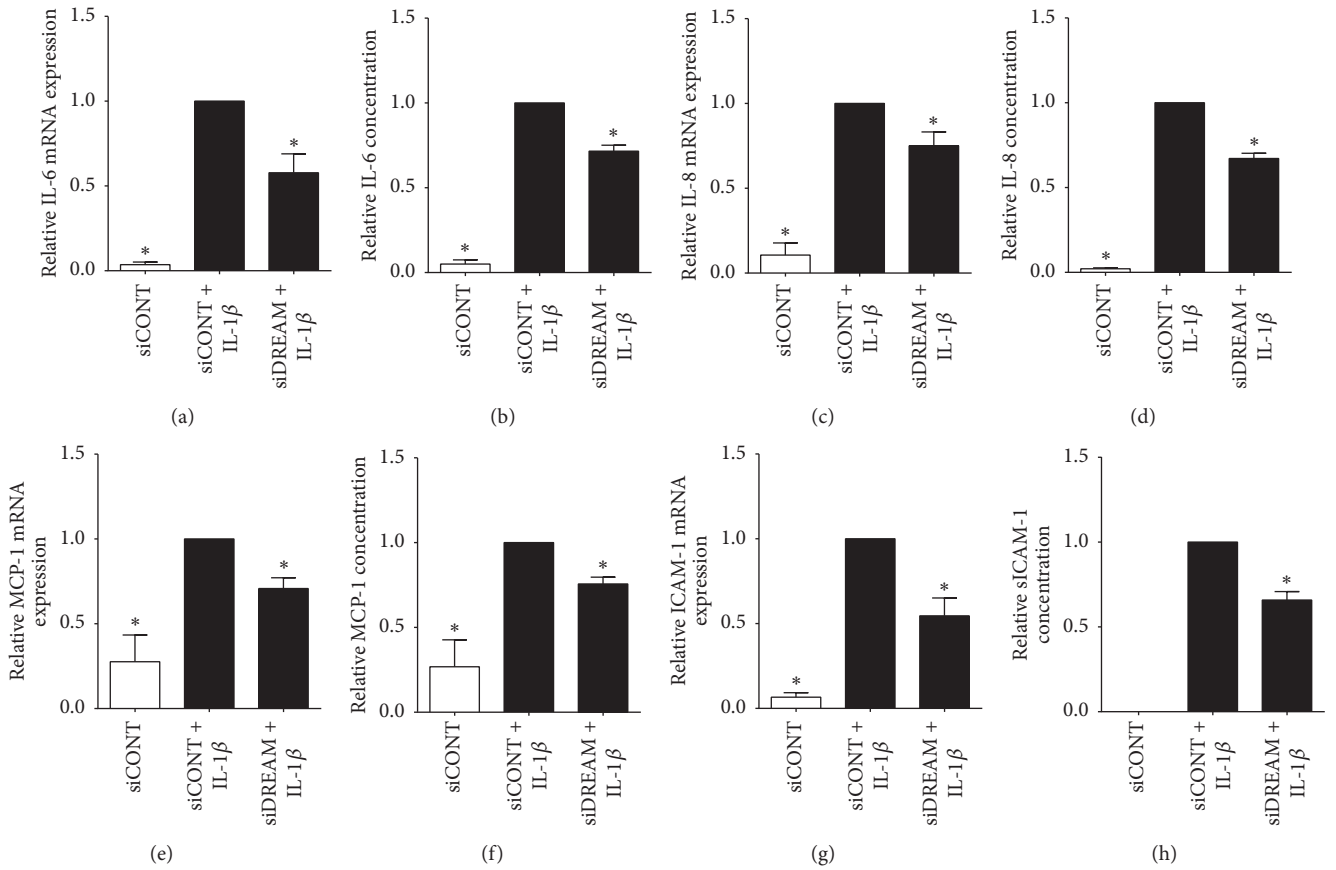

Figure 5: *Effect of siDREAM on proinflammatory cytokines, chemokines, and adhesion molecules in primary amnion cells*. Primary amnion cells were transfected with 50 nM siCONT or 50 nM siDREAM for 48 h and then treated with 100 pg/mL IL-1β for an additional 24 h ($n = 6$ patients). (a, c, e, g) IL-6, IL-8, MCP-1, and ICAM-1 mRNA expression were analysed by qRT-PCR. (b, d, f, h) The incubation media was assayed for concentration of IL-6, IL-8, MCP-1, and sICAM-1 by ELISA. For all data, fold change was calculated relative to IL-1β-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^*p \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells (one-way ANOVA).

NF-κB RelA transcriptional activity was significantly reduced in siDREAM transfected myometrial cells, suggesting that DREAM may regulate prolabour mediators via NF-κB signalling.

Increased DREAM expression is implicated in both physiological and pathological inflammatory states, including pain sensation (a hallmark of inflammation), Alzheimer's disease, and preeclampsia [27, 29, 32]. Inflammation is a common feature of human labour, with increased production of proinflammatory cytokines (such as IL-1β) by leukocytes infiltrating the myometrium, cervix, and fetal membranes [6, 7]. In this study, DREAM expression was similar between myometrium and fetal membranes obtained from nonlabouring and labouring women at term. On the other hand, at preterm, DREAM expression was significantly increased in fetal membranes from labouring women compared to nonlabouring women. This suggests that DREAM is not involved in the processes of healthy labour at term but is involved in the pathological activation of labour at preterm. Preterm labour is associated with increased inflammation in myometrium, fetal membranes, and amniotic fluid in the absence of infection, but also in an exaggerated manner in cases of infection [13]. We found that DREAM mRNA expression was significantly increased in preterm amnion with histological chorioamnionitis compared to amnion without histological chorioamnionitis. The fact that DREAM mRNA expression was increased in amnion with chorioamnionitis suggests that this increase may be caused by infection. Further studies are required to determine the role of proinflammatory and infectious stimuli in regulating DREAM expression. While it would be of great benefit to determine the expression of DREAM in myometrium from preterm deliveries with or without infection, obtaining such samples is extremely difficult. Notably, we only assessed DREAM mRNA expression; protein data are needed to verify these findings. Notwithstanding these limitations, the data collectively suggest that DREAM upregulation is more prevalent in states of heightened inflammation. Functional studies were then performed to determine whether DREAM regulates proinflammatory and prolabour mediators. For these studies, the proinflammatory cytokine IL-1β, two bacterial products (fsl-1 and flagellin), and one viral product (poly(I:C)) were

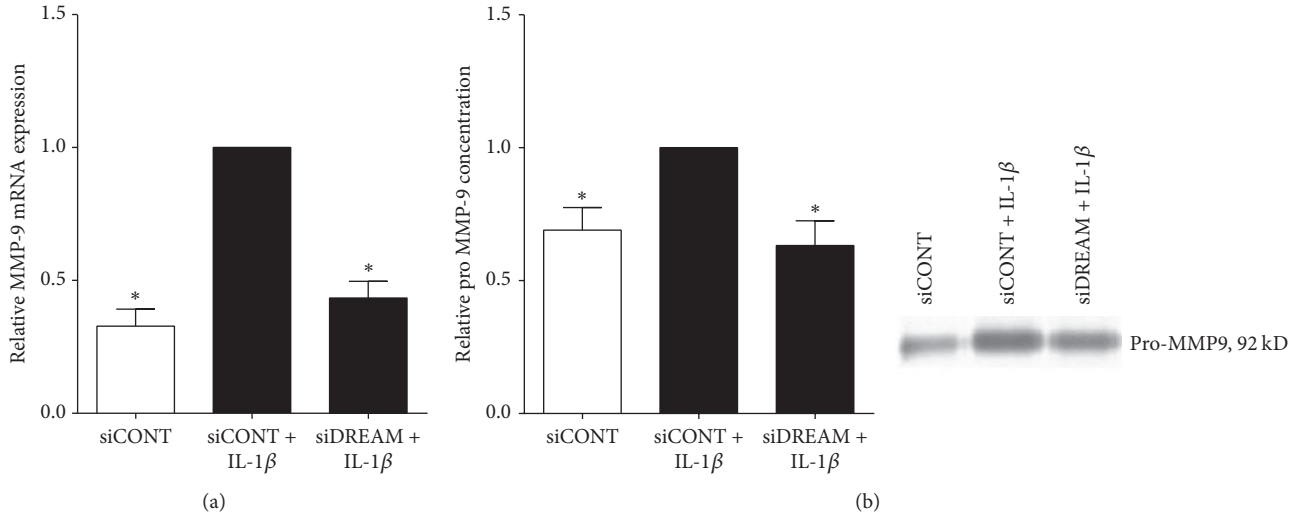

Figure 6: *Effect of siDREAM on the ECM degrading enzyme MMP-9 in primary amnion cells*. Primary amnion cells were transfected with 50 nM siCONT or siDREAM for 48 h and then treated with 100 pg/mL IL-1β for an additional 24 h (n = 6 patients). (a) MMP-9 mRNA expression was analysed by qRT-PCR. (b) The incubation media was assessed for pro-MMP-9 proteolytic activity using gelatin zymography. Representative zymogram from one patient is shown. For all data, fold change was calculated relative to IL-1β-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^{*}P \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells (one-way ANOVA).

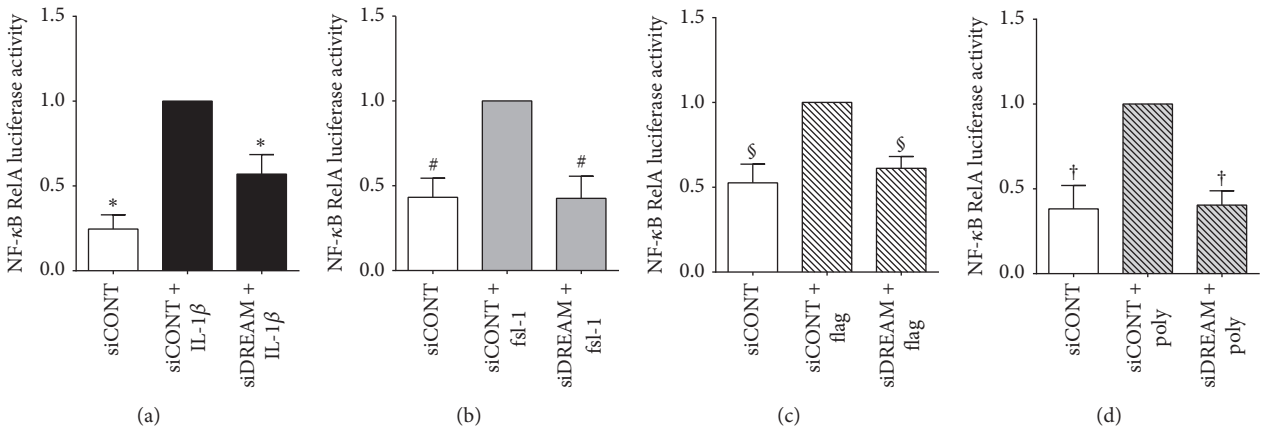

Figure 7: *Effect of siDREAM on NF-κB RelA transcriptional activity*. Primary myometrial cells were transfected with 300 ng/ml NF-κB RelA reporter construct for 6 h, transfected with 50 nM siCONT or siDREAM for 48 h, and then treated with (a) 100 pg/ml IL-1β, (b) 250 ng/ml fsl-1, (c) 1 μg/ml flagellin, or (d) 5 μg/ml poly(I:C) for an additional 24 h (n = 5-6 patients). Promoter activity was normalised to *Renilla* expression. Fold change was calculated relative to IL-1β-, fsl-1, and flagellin- or poly(I:C)-stimulated siCONT-transfected cells. Data are displayed as mean ± SEM. $^{*}P \leq 0.05$ versus IL-1β-stimulated siCONT-transfected cells; $^{\#}P \leq 0.05$ versus fsl-1-stimulated siCONT-transfected cells; $^{\S}P \leq 0.05$ versus flagellin-stimulated siCONT-transfected cells; $^{\dagger}P \leq 0.05$ versus poly(I:C)-stimulated siCONT-transfected cells (one-way ANOVA).

used to mimic inflammation associated with preterm labour [33, 41].

IL-1β is a proinflammatory cytokine released from infiltrating leukocytes in intrauterine tissues [7] that are central to the terminal pathways of human labour and delivery. Elevated IL-1β expression is found in the human myometrium, amnion, amniotic fluid, and cervix in association with term and preterm labour [6, 7, 42], while in intra-amniotic administration of IL-1β it induces preterm delivery in mice and rhesus monkeys [43, 44]. In intrauterine tissues, IL-1β has been shown to induce expression of chemokines, adhesion molecules, MMPs, and contractions associated proteins [10, 45–48]. In this study, siDREAM knockdown in primary myometrial and amnion cells was associated with significant decrease in IL-1β-induced expression and secretion of the proinflammatory cytokine IL-6, the chemokines IL-8 and MCP-1, and the adhesion molecule ICAM-1. Collectively, however, the results of this study indicate that DREAM is involved in the genesis of proinflammatory and prolabour mediators induced by IL-1β.

Activation of TLRs within intrauterine tissues is an important catalyst of preterm labour, with animal models identifying a role for TLR2 and TLR3 in particular. TLR2 ligation by bacterial products can induce preterm birth in

mice, while, conversely, mice lacking TLR2 demonstrate reduced expression of inflammatory and contractile genes as well as delayed timing of labour [49, 50]. There is also a synergy between TLR2 and TLR3, as combined stimulation using both agonists leads to induction of the inflammatory response and preterm labour in the mouse, caused by the alternate ligand [51]. In mice, advancing gestation is correlated with enhanced amniotic fluid expression of TLR2 and in humans TLR2 expression is increased in myometrium and fetal membranes in association with spontaneous term and preterm labour with evidence of chorioamnionitis [50, 52, 53]. Additionally, fsl-1, flagellin, and poly(I:C) (TLR2, TLR5, and TLR3 agonists, respectively) upregulate the expression and release of proinflammatory and prolabour mediators in myometrium and fetal membranes [21, 33, 41, 54]. DREAM has been shown to play an important role in regulating inflammation in response to infection. In a model of polymicrobial sepsis in DREAM-deficient mice, decreased IL-6, MCP-1, and ICAM-1 release was found in bronchoalveolar lavage fluid [24]. In support of these findings, our study demonstrates that siDREAM knockdown in primary myometrial cells associated with a significant decrease in fsl-1-, flagellin-, and poly(I:C)-induced expression and secretion of the proinflammatory cytokines IL-6, the chemokines IL-8 and MCP-1, and the adhesion molecule ICAM-1. This suggests that DREAM plays an important role in TLR signalling pathways associated with preterm labour.

Recent studies have identified DREAM as a regulator of NF-κB, a proinflammatory transcription factor critical to the synthesis of prolabour mediators [15, 16]. NF-κB signalling components, including RelA, have been identified in numerous gestational cells and tissues, with labour-associated increases in NF-κB signalling activity reported in myometrium, cervix, and amnion [55–58]. Decreased expression of multiple NF-κB signalling components has been demonstrated in lung vascular endothelial cells and neutrophils from DREAM-deficient mice [24, 25]. In our study, siDREAM knockdown was associated with a significant decrease in IL-1β-, fsl-1-, flagellin-, and poly(I:C)-induced NF-κB RelA transcriptional activity. Altogether, these findings suggest that, in human myometrium, DREAM is a mediator of the NF-κB signalling pathway, corroborating the potential of DREAM as a therapeutic target for the prevention of preterm labour.

Despite clinical interventions and extensive research, preterm birth rates continue to rise [59]. This may be due to an incomplete understanding of the mechanisms of human labour. This study suggests a role for DREAM in inflammation- and/or infection-induced preterm birth. DREAM mRNA expression is increased with preterm labour and in preterm amnion with histological chorioamnionitis, with loss-of-function studies suggesting that DREAM may regulate proinflammatory and prolabour mediators via NF-κB signalling in human myometrium and fetal membranes. Thus, inhibition of DREAM represents a novel therapeutic strategy for the prevention and management of preterm labour. Further studies are required to fully ascertain the role of DREAM in the processes of parturition.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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