Primary prevention with vaginal chlorhexidine before 16 weeks reduces the incidence of preterm birth: results of the Preterm Labor Prevention Using Vaginal Antiseptics study

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BACKGROUND: Preterm labor is one of the leading causes of perinatal death and is currently considered a syndrome with many causes. One of the most important causes of preterm birth is ascending infection from bacterial vaginosis. Chlorhexidine has proven to be effective against bacterial vaginosis and against bacterial biofilms without affecting gestation.

OBJECTIVE: We aimed to evaluate the effectiveness of a universal primary prevention strategy for preterm birth using intravaginal chlorhexidine applied before 16 weeks (Preterm Labor Prevention Using Vaginal Antiseptics study).

STUDY DESIGN: We performed a prospective observational study with 2 cohorts of pregnant women that were assigned either to prevention of preterm birth by means of intravaginal chlorhexidine (Cum Laude Chlorhexidine, chlorhexidine digluconate 0.2%) before 16 weeks (n=413), or to no treatment following the usual hospital protocol (n=704). Primary outcomes were the incidence of spontaneous preterm birth before 34 and 37 weeks; the incidence of preterm birth before 34 and 37 weeks, including inductions for premature rupture of membranes; and the incidence of preterm birth before 34 and 37 weeks, including any indication for termination of pregnancy. Both cohorts were compared using Mann-Whitney and Fisher tests. Finally, a multivariable analysis, including the odds ratio was performed, adjusting for clinical parameters, to evaluate the importance of the different determinants in the prediction of preterm birth.

RESULTS: In pregnancies treated with chlorhexidine, the incidences of spontaneous preterm birth; preterm birth, including induction for premature rupture of membranas; and preterm birth, including any indication for termination of pregnancy were at 34 and 37 weeks: 0% and 0%, 0.24% and 1.69, and 2.90% and 3.15%, respectively; whereas in nontreated pregnancies, these incidences were 9% and 11%, 12% and 23%, and 35% and 43%, respectively. According to the multivariable analysis, the incidence of preterm birth among women treated with chlorhexidine before 16 weeks was halved (Odds ratio, 0.52; $P<.05$).

CONCLUSION: Universal treatment with vaginal chlorhexidine before 16 weeks reduces the incidence of preterm birth, especially before 34 weeks.

Key words: bacterial vaginosis, chlorhexidine, labor onset, premature rupture of membranas, preterm birth, primary prevention, vaginal dysbiosis

Introduction

Preterm birth (PTB) is the world's leading cause of neonatal and childhood death. Its incidence and morbimortality are particularly important in low-income and middle-income countries,[1] but also in western countries, where it represents an important economic burden[2] and a major challenge for obstetrical and pediatric care.

PTB is considered a syndrome resulting from multiple etiologic pathways.[3] Among them, inflammation has emerged as the most plausible route for labor onset through the production of mediators that can be detected in amniotic fluid, cervical secretions, and maternal blood.[4–6] These molecules

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The authors report no conflict of interest.

Patient consent is not required because no personal information or details are included.

The trial was registered on ClinicalTrials.gov (Identifier: NCT05944094).

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Why was this study conducted?

Most prevention measures arrive too late to reduce preterm birth incidence, because they are implemented after inflammation has been triggered. In this work, we advocate for primary prevention to reduce bacterial overgrowth and avoid ascending infection.

Key findings

Universal treatment with chlorhexidine is likely to halve the incidence of preterm birth

What does this add to what is known?

Earlier studies have tried to lower the incidence of preterm birth by treating screen-positive cases with antibiotics and probiotics; however, the results have been poor. Conversely, we implemented a universal approach reducing the incidence of preterm birth by 50%.

may derive from minor infections distally located, such as the oral cavity in periodontitis,[7,8] or from minor infections, bacterial overgrowth, and dysbiosis proximally located in the vagina and cervix, such as bacterial vaginosis (BV).[8,9]

Recent reports have associated preterm premature rupture of membranes (PT-PROM) and PTB with BV, where the lactobacillus is replaced by an anaerobic/aerobic polymicrobial flora;[10–12] however, PTB prevention using clindamycin and metronidazole in patients positive for BV has proven to be controversial[13–22] in line with most secondary and tertiary prevention measures,[23–28] which seem to arrive too late to notably reduce PTB incidence (Figure 1). In addition, primary prevention has so far been limited to lifestyle changes and nutritional education,[29,30] but not to the reduction of bacterial overgrowth and vaginal dysbiosis. Consequently, the appropriate strategy for the prevention of PTB has yet to be discovered.

Chlorhexidine (CLX) is a cheap, effective,[31] and innocuous treatment that has long been tested to be effective against vaginal vaginosis in nonpregnant women[32] and is also efficacious against biofilms produced by bacteria[33,34] without affecting gestation (US Food and Drug Administration category B).[35,36] This study aimed to evaluate the effectiveness of universal intravaginal CLX as a primary strategy to prevent PTB.

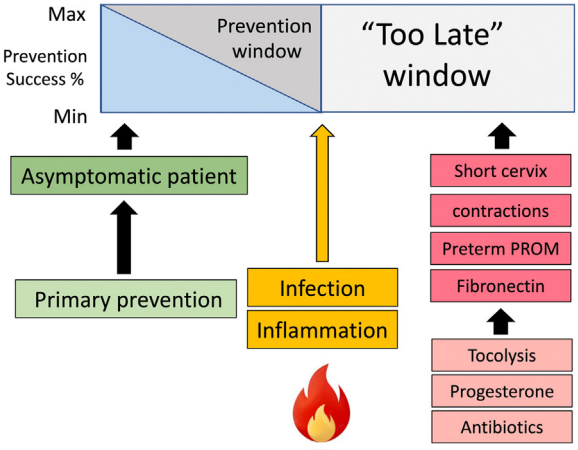
Material and Methods

This was a prospective observational study with 1117 pregnant women attending the general obstetrics clinic at La Fe Maternity Hospital aimed to evaluate the effect of intravaginal CLX treatment on the incidence of PTB before 34 and 37 weeks (Preterm Labor prevention Using Vaginal Antiseptics [PLUVA] study; ClinicalTrials.gov Identifier: NCT05944094).

Inclusion criteria were singleton normal pregnancies with no previous history of preterm birth or cervical surgery aiming to deliver in our maternity hospital. Exclusion criteria were previous PTB, previous cerclage, multiple pregnancies, subsequent abortion, chromosomal or morphologic anomalies, loss of follow-up, and final delivery in a different center. The study started in 2019 and was completed 4 years later in 2023 when the planned number of cases was collected. The sample size was calculated in 848 cases (424 in each group) to demonstrate a 30% reduction in the rate of PTB according to previous information and assuming an alpha value of.05 and a beta value of.2.

All patients underwent an initial ultrasound examination between 6+0 and 15+6 weeks' gestation (usually at 11 weeks), which included an evaluation of the embryo or fetus vitality and a measurement of the crown rump length with correction of the estimated delivery date when appropriate. Ultrasound examinations were performed by the first author, a certified expert in obstetrical ultrasound by the Spanish Society of Obstetrics and Gynecology, using a Samsung (Samsung-Medison, Gangdong-gu, Seoul, Republic of Korea) Sonoace R7 ultrasound machine equipped with a multifrequency convex probe.

FIGURE 1
Rationale of primary prevention



Thus far, most actions aimed to reduce the incidence of preterm birth have been implemented too late in the natural history of the disease. However, to be effective, actions should be performed when the patient is asymptomatic, and before the onset of infection and inflammation.

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There was no selection of cases. Recruiting calendar was planned according to the days the authors were in the early pregnancy clinic. Even numbers in the calendar were allocated to treatment and odd numbers were allocated to nontreatment following the usual hospital protocol. The reason for the higher number of cases in the nontreatment branch (n=701 vs n=413) was explained by the reluctancy to accept the treatment; thus the number of cases collected in the even days was slightly lower. Because the cases and controls were not properly randomized, we classified the study simply as observational.

Antiseptic treatment aimed to reduce potential bacterial overgrowth consisted in 10 days (1 box) of Cumlaude CLX vaginal ovules (chlorhexidine digluconate 0.2%, price 12€, Laboratorios Cumlaude-Dermofarm, Rubí, Barcelona, Spain) starting always between 9 +0 and 16+0 weeks. Pregnancies were followed until the end of pregnancy, when all data related to labor were collected, as well as all events related to PTB, such as onset of contractions, cervical shortening, and PT-PROM, regardless of the final gestational age (GA) at delivery. Unfortunately, we were unable to fully ensure treatment compliance because this was performed privately at home. Outcome measures were spontaneous PTB before 34 and 37 weeks only including spontaneous onset of labor; PTB before 34 and 37 weeks also including inductions for PT-PROM; and PTB before 34 and 37 weeks, including any indication for termination of pregnancy.

All fetuses were managed according to the hospital protocol and their progression in labor; as per hospital protocol, PT-PROM were induced not later than 34+6 weeks, and preterm contractions were treated with tocolytic agents and corticoids to delay delivery and allow lung maturation and neuro-prophylaxis.

Statistical analysis

Descriptive statistics were performed evaluating maternal age; parity; GA at examination in weeks; GA at delivery in weeks; interval between ultrasound and delivery; birthweight (BW); BW centile; fetal gender; onset of labor (elective cesarean delivery, induction of labor, and spontaneous onset of labor); mode of delivery (cesarean delivery for abnormal cardiotocography, failure to progress and elective indications, assisted delivery, and spontaneous delivery); Apgar scores at 5 minutes; neonatal cord arterial pH; and whether the newborn was admitted to the maternity ward, neonatal ward, or neonatal intensive care unit. Continuous variables were presented as mean and standard deviations, median, and interquartile range, whereas categorical variables were presented as absolute numbers and relative frequencies.

Characteristics between both cohorts were compared with Mann-Whitney and Fisher tests. Finally, to assess the validity of the results and ensure consistency, an additional multivariable analysis was performed, adjusting for some plausible clinical parameters (maternal age, parity, weight, height, smoking, and fetal sex) to evaluate the odds ratio (OR) of the different determinants in the prediction of PTB. The area under the curve (AUC) and the negative predictive power of the model were described. Finally, PTB incidence was calculated for specific groups selected according to the multivariable analysis result.

Statistical analysis and graphs were done using GraphPad Prism, Mac version 9.0.1 (GraphPad Software, Boston, MA 02110, USA), and Stat Plus Mac Pro version 8.0.1.s (AnalystSoft Inc, Walnut, CA 91789). Significance was considered at $P<.05$. Permissions were obtained from La Fe hospital review board and from the Valencian Autonomic Government health authorities (reference: PLUVA; date February 4, 2021). Written informed consent was obtained to participate in the study.

Results

In Table 1, we describe the characteristics of treated and nontreated cases. Apart from the longer interval of examination-to-delivery in the treated group ($P<.05$), no other difference was observed, proving that both cohorts were homogeneous and comparable.

In Table 2 and Figure 2, we compare the incidence of PTB in both cohorts. The incidences of spontaneous PTB before 34 and 37 weeks, respectively, were 0% (0/413) and 1.69% (7/413) in treated pregnancies, and 1.28% (9/704) and 3.27% (23/704) in nontreated pregnancies ($P<.05$, P=not significant [NS]). The incidences of spontaneous PTB before 34 and 37 weeks, respectively, including induction for premature rupture of membranes, were 0% (0/413) and 2.9% (12/413) in treated pregnancies, and 1.56% (11/704) and 4.97 % (35/704) in nontreated pregnancies ($P<.01$, P=NS). Finally, the incidences of spontaneous PTB before 34 and 37 weeks, respectively, including any indication for termination of pregnancy were 0.24% (1/413) and 3.15% (13/413) in treated pregnancies, and 1.7 % (12/704) and 6.11% (43/704) in nontreated pregnancies ($P<.05$, all). Pregnancy termination for other reasons was indicated in 9 cases (1 in the treated group). Most of the indications were related with growth disorders such as preeclampsia, intrauterine growth restriction, and smallness for GA (Table 3).

In Table 4, we show the multivariable analysis for the prediction of PTB before 37 weeks, in which CLX treatment was adjusted for several pregnancy characteristics to evaluate the true importance of these variables for the prediction of PTB. Only CLX treatment and fetal sex were significant. The OR of the former was 0.52, whereas the OR of fetal sex (male) was 1.85. This means that the incidence of PTB among women treated with CLX was halved, whereas it nearly doubled among women gestating a male fetus. The AUC of the model was low (0.63); however, it presented a significant negative predictive power of 95%.

In accordance with these ORs, in Figure 3, we present the incidence of PTB before 37 weeks in 4 selected groups. As earlier indicated, PTB occurred in 6.11% (43/704) of the general population (pregnancies without CLX treatment), and in 3,15% (13/413)

TABLE 1
Descriptive statistics of the 2 cohorts according to the treatment with chlorhexidine

Parameter	Chlorhexidine (n=413)		No chlorhexidine (n=704)		
	Mean (SD)	Median (first–third quartile)	Mean (SD)	Median (first–third quartile)	*P* value
Maternal age (y)	31.9 (5.4)	32 (28–36)	31.9 (5.7)	32.0 (29–36)	NS
Gestations	2.1 (1.3)	2 (1–3)	2.2 (1.3)	2 (1–3)	NS
Parity	0.66 (0.86)	0 (0–1)	0.68 (0.86)	0.5 (0–1)	NS
Maternal prepregnancy weight (kg)	64.7 (12.8)	63 (55–71)	64 (13.3)	62 (55–71)	NS
Maternal height (cm)	162.9 (6.5)	163 (158–167)	162.7 (6.6)	163 (159–167)	NS
Maternal body mass index	24.3 (4.4)	23.6 (21.1–26.3)	24.3 (6)	23.2 (20.7–26.7)	NS
GA at examination	11.3 (1.2)	11.1 (10.4–12.3)	11.4 (1.4)	11.9 (10.4–12.4)	NS
Crown-rump-length at examination	47.9 (16.1)	48 (35.2–61)	49.5 (16.7)	52.5 (35–62)	NS
GA at delivery	39.5 (1.3)	39.7 (38.8–40.4)	39.3 (1.7)	39.7 (38.7–40.4)	NS
Interval examination-delivery	197.4 (12.4)	198 (191–205)	195.3 (15.1)	196 (187–205)	<.05
BW (g)	3328 (463)	3340 (3005–3645)	3266 (508)	3265 (2980–3600)	NS
BW centile[a]	52.1 (30.8)	51 (24–81.5)	49.5 (31.1)	48 (22–77)	NS
Apgar at 5 min	9.8 (0.49)	10 (10–10)	9.8 (0.52)	10 (10–10)	NS
Arterial cord pH	7.26 (0.11)	7.26 (7.21–7.30)	7.26 (0.07)	7.27 (7.21–7.31)	NS
		N (%)		N (%)	
Nulliparous		216 (52.3)		352 (50)	NS
Sex male		201 (48.7)		350 (49.7)	NS
Apgar 5 min <7		2 (0.48)		3 (0.43)	NS
Arterial cord pH <7.10		14 (3.4)		17 (2.4)	NS
Smoking		44 (10.6)		99 (14)	NS
SGA (BW<10th centile)		40 (9.7)		79 (11.2)	NS
Onset of labor					
Spontaneous onset of labor		196 (47.4)		331 (47)	NS
Induction of labor		177 (42.8)		291 (41.3)	NS
Elective cesarean delivery		40 (9.7)		82 (11.6)	NS
Through labor					
Spontaneous vaginal delivery		249 (60.3)		429 (60.1)	NS
Assisted vaginal delivery		58 (14.0)		99 (14)	NS
Cesarean delivery abnormal CTG		23 (5.6)		38 (5.4)	NS
Cesarean delivery for failure to progress		43 (10.4)		56 (7.9)	NS
Elective cesarean delivery		40 (9.7)		82 (11.6)	NS
Neonate destiny					
Maternal ward		399 (96.6)		667 (94.7)	NS
Neonatal special or intensive care unit		14 (3.4)		37 (5.2)	NS

BW, birthweight; *CTG*, cardiotocography; *GA*, gestational age; *SD*, standard deviation; *SGA*, small for gestational age (<10th percentile).

[a] BW centiles according to local population references (Hospital Clinic de Barcelona, Spain population references).

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TABLE 2
Incidence of PTB before 34 and 37 weeks in treated and untreated patients

Type of preterm birth onset	Chlorhexidine (N=413) n (%)	No chlorhexidine (N=704) n (%)	*P* value
Preterm birth (<34 wk), including only spontaneous onset of labor[a]	0 (0)	9 (1.28)	<.05
Preterm birth (<34 wk), also including induction after PRM[b]	0 (0)	11 (1.56)	<.01
Preterm birth (<34 wk), including any indication for termination of pregnancy	1 (0.24)	12 (1.70)	<.05
Preterm birth (<37 wk), including only spontaneous onset of labor[a]	7 (1.69)	23 (3.27)	NS
Preterm birth (<37 wk), also including induction after PRM[b]	12 (2.90)	35 (4.97)	NS
Preterm birth (<37 wk), including any indication for termination of pregnancy	13 (3.15)	43 (6.11)	<.05

PRM, premature rupture of membranes; *PTB*, preterm birth.

[a] Spontaneous onset of labor included both, initiation of contractions after premature rupture of membranes and initiation of contractions before premature rupture of membranas; [b] After 34 weeks, all pregnancies with PRM were induced.

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of pregnancies treated with CLX. However, PTB occurred in 2.27% (16/704) of pregnancies with a female fetus and only in 1.21% (5/413) of treated pregnancies with a female fetus. This last combination presented the lowest risk for PTB. No adverse effects such as burning, itching, etc., were reported by any of participating treated women.

Discussion

Principal findings

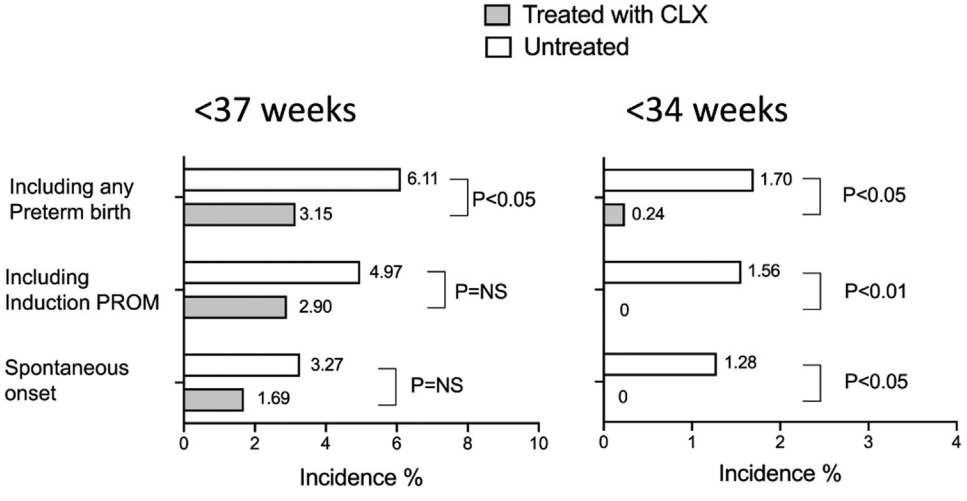
Patients undergoing universal primary prevention with intravaginal CLX reduce the incidence of PTB; this reduction is clearer in early PTB before 34 weeks.

Results

Our results suggest that universal treatment with CLX might be important to diminish the incidence of PTB. CLX is cheap and easy to administer. In addition, it presents an excellent safety profile,[35,36] and is as effective as metronidazole and clindamycin for the treatment of BV.[37] Moreover, vaginal CLX administration before cesarean delivery reduces the risk of endometritis, puerperal fever, wound infection, and transmission of Group B Streptococcus to the fetus, thereby reducing neonatal sepsis and mortality.[38,39]

Earlier studies have tried to lower the incidence of PTB associated with BV[40] by treating only screen-positive cases with metronidazole or clindamycin. The results of such an approach have been poor and controversial.[17,41–43] Others, however, have advocated for a universal treatment with probiotics, reporting a reduction of PTB.[44,45] In this study, we have proposed a mixed approach, using CLX to reduce bacterial

FIGURE 2
Incidence of preterm birth in treated and nontreated pregnancies



PTB, preterm birth.

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TABLE 3
Preterm birth deliveries for other medical indications

Case	CLX	Diagnosis	Week of delivery	Onset of labor	Way of delivery	BW (g)	BW centile	Apgar 5′	A pH	Sex	Baby destiny
1	No	Very short intercesarean interval	36,00	Elective cesarean delivery	Elective cesarean delivery	2510	27	10	7.27	Male	Neonates
2	No	Preeclampsia	36,29	Elective cesarean delivery	Elective cesarean delivery	2040	1	10	7.32	Male	Neonates
3	No	Intrauterine growth restriction	36,57	Induction of labor	Spontaneous vaginal delivery	2060	1	9	7.22	Male	Neonates
4	No	Third trimester metrorrhagia	36,71	Induction of labor	Spontaneous vaginal delivery	2950	66	10	7.33	Male	Maternal ward
5	No	Polyhydramnios	36,71	Elective cesarean delivery	Elective cesarean delivery	4070	100	10	7.31	Male	Neonates
6	No	Placenta previa	36,86	Elective cesarean delivery	Elective cesarean delivery	2850	49	10	7.27	Male	Maternal ward
7	No	Small for gestational age	36,86	Induction of labor	Spontaneous vaginal delivery	2270	4	10	7.22	Female	Maternal ward
8	No	Intrauterine growth restriction	32,29	Elective cesarean delivery	Elective cesarean delivery	1320	0	10	7,30	Female	Maternal ward
9	Yes	Preeclampsia	31,86	Elective cesarean delivery	Elective cesarean delivery	1090	0	10	7,30	Male	Neonates

BW, birthweight; *CLX*, chlorhexidine treatment.

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overgrowth but universally to reach the entire population.

Clinical implications

Time is a powerful ally in the prevention of diseases. Accordingly, simple measures early implemented in the natural history of morbid conditions can frequently achieve more goals than complicated procedures put in place after the morbid process has been triggered. Perhaps the clearest example is handwashing for the prevention of infections, which has made soap the most cost-effective drug ever used. The natural history of PTB and PT-PROM mimics that of infectious-inflammatory procedures[46–48] in which most resources have been applied to reduce the severity of the morbid condition (PTB) after it has been triggered, applying tocolysis, antibiotics, or even surgery (cerclage)[23–28] by means of different approaches that probably come too late to succeed.

Consequently, it has become increasingly apparent that actions to prevent PTB should take place earlier during the natural history of the disease[46–48] by using primary prevention. Unfortunately, primary prevention has so far addressed tangential issues such as lifestyle and nutrition[29,30] but has not tackled vaginal dysbiosis. This scenario prompted us to investigate the possibility of decreasing PTB by reducing vaginal bacterial overgrowth.

Research implications

CLX is effective against vaginal dysbiosis and against biofilms[33,34] produced by bacteria without affecting gestation. Therefore, the rationale of the CLX application lies in the reduction of bacterial overgrowth that, in a proportion of susceptible patients (lower immunity or special anatomic conditions)[49–51] might cause an ascending infection, triggering inflammation and PTB.[52–54] Our results open a new path for research in which secondary and tertiary prevention may be substituted by a more effective primary prevention aimed at stopping the natural history of PTB much earlier before its onset.

TABLE 4
Multivariable model for the prediction of preterm birth (any cause) before 37 weeks

Parameter	β-Coefficient	SE	OR (95% CI)	*P* value
Age	0.00722	0.02492	1.00724 (0.95923–1.05766)	NS
Parity	−0.08534	0.17705	0.91820 (0.64898–1.29911)	NS
Weight	−0.00134	0.01030	0.99867 (0.97870–1.01904)	NS
Height	−0.03170	0.02065	0.96880 (0.93038–1.00881)	NS
Smoking	0.15467	0.39744	1.16727 (0.53563–2.54378)	NS
Chlorhexidine treatment	−0.64568	0.32503	0.52430 (0.27727–0.99142)	<.05
Fetal Sex (male)	0.61459	0.28853	1.84890 (1.05030–3.25472)	<.05
Intercept	1.92581			

AUC=0.63, *P* value=.0014, negative predictive power 95%.

AUC, area under the curve; *CI*, confidence interval; *OR*, odds ratio; *SE*, standard error.

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Strengths and limitations

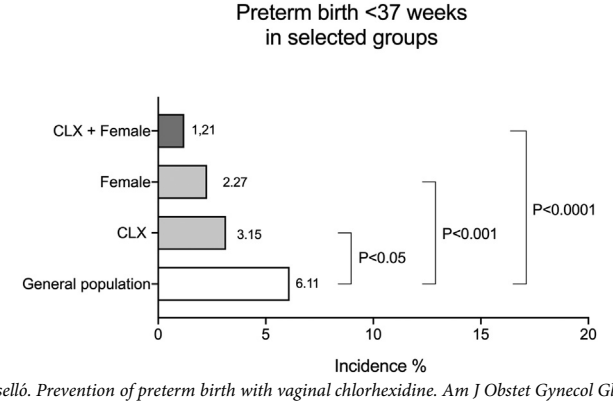
The main strengths are: the application of universal primary prevention, the robust statistical approach using multivariable analysis and multiple comparisons between different PTB groups with specific characteristics, and the fact that the rate of PTB <37 weeks in the control population (6.1%) was very similar to the authors' national rates of PTB. Another strength is that ascending intrauterine infection seems to be causative of PTB particularly in the very preterm period (<34 weeks). So, if the intervention was efficacious, it would be quite useful, particularly in low-income countries with high rates of early spontaneous PTB due to infection.

Conversely, limitations include the moderate number of cases and the impossibility to ensure the compliance of the CLX treatment, which was applied privately at home.

Conclusions

Universal treatment with vaginal CLX before 16 weeks reduces the incidence of PTB, especially before 34 weeks. ■

FIGURE 3
Incidence of preterm birth considering CLX treatment and fetal sex



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