Disruption of Circadian Rhythms of preterm PA and PPROM

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Outline

1. **Background**
   - Introduction
   - The hypothesis
   - Justification
   - Objectives

2. **Methods**
   - Data, settings and sample size
   - Statistical analysis: Circular Time and Fourier Transformation

3. **Results**
   - Results
   - Replication: NCPP study

4. **Discussion**
   - Main findings
   - Limitations
   - Interpretation
   - Conclusions

**HSPH-Department of Epidemiology**

Disruption of Circadian Rhythms of preterm PA and PPROM
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Background

- Globally, **15 million** preterm deliveries are estimated to take place every year.
- Around **1 million** children die each year due to complications of prematurity and placental abruption (PA).
- PA is a significant contributor to maternal mortality and it occurs in **1%** of pregnancies worldwide.
The time of onset of preterm PA and extremely and very PPROM cases may be characterized by a disrupted circadian pattern associated with fetal immaturity.
The disruption of circadian rhythms driven by fetal immaturity may help to explain tocolytics failure to prevent delivery of very and extremely PPROM cases, and therefore could help to orientate the clinical practice.

The time of the onset of PPROM and PA cases has not been modeled with the most appropriate methodology. Therefore, an appropriate methodological approach could help to test more consistently the hypothesis.
The disruption of circadian rhythms driven by fetal immaturity may help to explain tocolytics failure to prevent delivery of very and extremely PPROM cases, and therefore could help to orientate the clinical practice.

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Figure 1. Number of cases versus time of day of onset of labour in twin pregnancies: at 24–36 weeks of gestation and at 37–40 weeks of gestation.

\textit{HSPH-Department of Epidemiology} Disruption of Circadian Rhythms of preterm PA and PPROM
The objectives

1. **To model**, with the appropriate methodology (time series theory and circular time), the time of onset of PPROM and PA cases.

2. **To evaluate** the presence of circadian rhythms for PPROM and PA cases.
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3. **To examine** the extent to which the circadian rhythms vary based on gestational age at onset.
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METHODS

Data, setting and sample size.
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- A total of **232** PPROM and **163** PA singleton spontaneous cases were identified at three hospitals in Lima, Peru, between January 2009 and July 2010.

- **Exclusions:** Multiplicity, labor induction and/or artificial rupture of membranes and gestational age unknown.
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Methods: Case definition

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METHODS
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Time in a linear scale

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Or almost never!
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But often the linearity assumption is good enough.
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Time in circular scale

- Circular time: trigonometric models or cosinor models
- Polynomials, trigonometric polynomials, fractional polynomials
- Splines, restricted cubic splines, smoothed splines...
Methods

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**Methods general overview**

**Circular Time**

- **Assessing periodicity**: Fourier Times Series Transformation (Periodogram).

- **Circumference of Radius 1**

- **Statistical analysis**: Circular Time and Fourier Transformation
Methods general overview

Circular Time

Circular time

- **Assessing periodicity**: Fourier Times Series Transformation (Periodogram).
- **Describing periodicity**: Data reduction.
Methods general overview

**Circular Time**

- Describing periodicity: Data reduction.
- Modeling periodicity: Trigonometric predictors with sine and cosine terms (Trigonometric regression or cosinor model).
Methods general overview

Circumference of Radius 1

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- Describing periodicity: Data reduction.
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Circular time modeling assumptions

- Sinusoidal pattern
- Stationary time series
Methods general overview

Circular Time

-1 0 1

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Circular time

- Describing periodicity: Data reduction.
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Circular time modeling assumptions

Sinusoidal pattern ——— Stationary time series

Disruption of Circadian Rhythms of preterm PA and PPROM
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\[ \sin(0^\circ) = 0.0 \]
\[ \cos(0^\circ) = 1.0 \]
RESULTS AND COMMENTS ON METHODS
Results: Describing/assessing periodicity

Describing the data: Data reduction

- **Aggregated number** of cases and percentages by hours and the four segments of the day.
- **Prevalence ratios** (PRs) of the aggregated number of cases by segments of the day with night as reference (PRs were derived using a generalized linear Poisson model with link log and scaled error standards to account for over-dispersion).
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Results: assessing periodicity

Kernel smoother and Fourier Transformation

1. We used a non-parametric Kernel density estimation to estimate the probability density function of PPROM and PA cases over time (24 hours).

2. We used a periodogram (Fourier transformation: sine and cosine functions) to identify any periodicity: higher frequencies in the distribution of the observed data.
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Assessing periodicity: distributions and Periodogram

Results

Replication: NCPP study

Background

Methods

Results

Discussion

Assessment of periodicity: distributions and Periodogram

Preterm PROM

Placental Abruption

Kernel density

Distribution of the number of onsets in %

Time in 24 hours

Sample spectral density function (Preterm PROM), N=232

Sample spectral density function (Placental Abruption), N=163

Log Periodogram of the number of onsets

Frequency

Disruption of Circadian Rhythms of preterm PA and PPROM
Testing periodicity: Cosinor models

Modeling the time of onset of PPROM AND PA: Cosinor models
The cosinor Model: trigonometric regression

The Cosinor model: Generalized Linear Model

\[ g(Y)_t = c \cos(w_t) + s \sin(w_t) \]
The cosinor Model: trigonometric regression

Cosinor

The **Cosinor** model: Generalized Linear Model

\[ g(Y)_t = c \cos(w_t) + s \sin(w_t) \]

\[ t = 1, \ldots, 24. \]
Scaling time to a circular scale.
We compute \( w_t \) as follow:

Scaling time to a circular scale.

Where the Amplitude is derived as follows:

\[ A = \sqrt{c^2 + s^2} \]

\( A \geq 0 \)

Then we test the following Null Hypothesis

The amplitude is \( A = 0 \)

Using the following test:

\[ Z = \frac{n\bar{A}^2}{\phi} \]

\[ \phi = \sqrt{\frac{\chi^2}{df}} \]
The cosinor Model: trigonometric regression

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Deriving the amplitude and testing the statistical significance of the circadian rhythm

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Amplitude

One cycle per $2\pi$ units of time

- **sine**
- **cosine**

TIME (x)
Preterm Premature Rupture of Membranes in Lima, Peru, n= 232

A Moderate Preterm (32 to <37 gestation weeks)
N=154(66.4%)

B Extremely-very Preterm (<32 gestation weeks)
N=78(32.6%)

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Circadian Test P-value <0.001
Circadian Test P-value 0.259
Placental Abruption cases, in Lima, Peru, n= 163

A Term cases, N= 77 (47.2%)

B Preterm cases, N= 86 (52.8%)

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Disruption of Circadian Rhythms of preterm PA and PPROM
Placental Abruption cases, in Lima, Peru, n= 163

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Circadian Test P-value: 0.067

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NCPP REPLICATION

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Description of the NCCP study

The US National Collaborative Perinatal Project (NCPP)

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- The NCPP was specifically designed to identify determinants of cerebral palsy and other neurological defects.
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PA ≥ 37 gestation weeks

NCCP TERM PA cases
TERM Placental Abruption NCPP, n= 231

Term spontaneous, singleton PA cases, (N=231, >=37 gestation weeks)

Circadian Test p-value <0.05

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DISCUSSION

DISRUPTION OF CIRCADIAN RHYTHMS OF PRETERM PA AND PPROM
Main findings

Disruption of Circadian Rhythms

- We found a **significant diurnal circadian pattern** in the timing of the onset of PPROM cases and some evidence among term PA cases in Lima, Peru.
- We did **not find evidence** of a circadian pattern among cases of very and extremely PPROM and PA.
Main findings

**Disruption of Circadian Rhythms**

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LIMITATIONS

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Disruption of Circadian Rhythms of preterm PA and PPROM
Limitations

- **Measurement error** due to maternal self-reports starting signs and symptoms.
- High specificity for severe cases of PPROM and PA though compromise in sensitivity for mild PA and PPROM cases.
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- Need of more replication studies in other geographic regions and populations.
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Disruption of Circadian Rhythms of preterm PA and PPROM
Our findings are consistent with previous evidence and may be explained by a biological mechanism (HPA-axis).

The fetal immaturity of the HPA-axis may account for the absence of circadian rhythms in extremely preterm deliveries.
Interpretation

- Our findings are **consistent** with previous evidence and may be explained by a **biological mechanism (HPA-axis)**.
- The fetal immaturity of the HPA-axis may account for the **absence of circadian rhythms** in extremely preterm deliveries.
- Other mechanisms driven by **infection (chorioamnionitis) and inflammation** may explain the triggering of parturition in extremely and very preterm cases.
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- The fetal immaturity of the HPA-axis may account for the absence of circadian rhythms in extremely preterm deliveries.
- Other mechanisms driven by infection (chorioamnionitis) and inflammation may explain the triggering of parturition in extremely and very preterm cases.
Conclusions

- The time of onset of **very PPROM and preterm PA cases** is characterized by a **disrupted circadian pattern**.
- This disrupted circadian pattern underlies **other pathological mechanisms** associated with the time of onset of PPROM and PA case rather than a normal biological circadian rhythm.
Conclusions

- The time of onset of very PPROM and preterm PA cases is characterized by a disrupted circadian pattern.
- This disrupted circadian pattern underlies other pathological mechanisms associated with the time of onset of PPROM and PA case rather than a normal biological circadian rhythm.
- The disrupted circadian pattern driven by fetal immaturity may help to explain tocolytic failure to prevent delivery of very and extremely PPROM cases.
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- This disrupted circadian pattern underlies other pathological mechanisms associated with the time of onset of PPROM and PA case rather than a normal biological circadian rhythm.
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Conclusions

- The time of onset of very PPROM and preterm PA cases is characterized by a disrupted circadian pattern.
- This disrupted circadian pattern underlies other pathological mechanisms associated with the time of onset of PPROM and PA case rather than a normal biological circadian rhythm.
- The disrupted circadian pattern driven by fetal immaturity may help to explain tocolytic failure to prevent delivery of very and extremely PPROM cases.
References

Some important references


Acknowledgments

- **Professor Michelle A. Williams**. Chair of the Epidemiology Department, Harvard School of Public Health.
- **Dr. Bizu Gelaye**, Director of the MIRT program, Epidemiology Department, Harvard School of Public Health.
- **Rose Traveling Fellowship** Program in Chronic Disease Epidemiology and Biostatistics.
- **Dr. Unnur Valdimoarsdottir**, Center of Public Health Sciences, Faculty of Medicine, University of Iceland, Reykjavik, Iceland.
- **Dr. Sixto Sanchez**, AC. PROESA and the Instituto Nacional Materno Perinatal, Lima, Peru.
Thank You

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Alhambra: Granada, Spain
Methods: Case definitions ICD-10

Case definition based on

- **ICD-10 O42** (PPROM) and **O45** (PA).
- **ICD-10-O42** (PPROM): Spontaneous tearing of the membranes surrounding the fetus any time before the onset of obstetric labor prior 37 gestation weeks. Rupture of membranes will show pooling of fluid in the vagina or leakage of fluid from the cervix.
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<table>
<thead>
<tr>
<th>Time in 24h</th>
<th>Preterm PROM</th>
<th></th>
<th></th>
<th>Placental Abruption</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>01h:00’</td>
<td>10</td>
<td>4.3</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>02h:00’</td>
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<td></td>
<td>14</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>03h:00’</td>
<td>11</td>
<td>4.7</td>
<td></td>
<td>4</td>
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<td></td>
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<td>11h:00’</td>
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<tr>
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<tr>
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<tr>
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<td>1.3</td>
<td></td>
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Table: Counts of preterm PROM and PA onsets by hour per day, in Lima, Peru, 2009-2010 (Preterm PROM, n=232 and Placental Abruption, n= 163)
<table>
<thead>
<tr>
<th>Time in 24h</th>
<th>Preterm PROM</th>
<th>Placental Abruption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>01h:00'</td>
<td>10</td>
<td>4.3</td>
</tr>
<tr>
<td>02h:00'</td>
<td>6</td>
<td>2.6</td>
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### PPROM onsets

Distribution of preterm PROM onsets by segment of the day and gestation weeks, in Lima, Peru, 2009-2010 (Preterm PROM, n=232)

<table>
<thead>
<tr>
<th>Segments of the day</th>
<th>Preterm Premature Rupture of Membranes (n= 232)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(≥32-&lt;37 weeks, n=154)</td>
</tr>
<tr>
<td></td>
<td>n(%)</td>
</tr>
<tr>
<td>Night</td>
<td></td>
</tr>
<tr>
<td>01h:00' to 06h:00'</td>
<td>50(32.5)</td>
</tr>
<tr>
<td>Morning</td>
<td></td>
</tr>
<tr>
<td>07h:00' to 12:00'</td>
<td>56(36.4)</td>
</tr>
<tr>
<td>Afternoon</td>
<td></td>
</tr>
<tr>
<td>13h:00' to 18h:00'</td>
<td>25(16.2)</td>
</tr>
<tr>
<td>Evening/night</td>
<td></td>
</tr>
<tr>
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* PR: Prevalence Ratio

---

**Disruption of Circadian Rhythms of preterm PA and PPROM**
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<tr>
<td></td>
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<td></td>
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* PR: Prevalence Ratio
Assessing Periodicity: Periodogram

Number of cycles in $2\pi$ time

- The periodogram $I(w_j)$ is always positive, and it will be larger at frequencies that are strongly represented in the data.
- Therefore the number of time points needed to complete a cycle of $2\pi$ could be computed as the inverse of the Fourier frequency using:

$$1/f_j = \frac{2\pi}{w_j}$$

Formulae

$$I(w_j) = \frac{2}{n}(\hat{C}^2 + \hat{S}^2) \quad j = 1, \ldots n/2$$

$$\hat{C}^2 = 2 \sum_{t=1}^{n} y_t \cos(w_j t)/n,$$

$$\hat{S}^2 = 2 \sum_{t=1}^{n} y_t \sin(w_j t)/n,$$
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\]

\[
\hat{S}^2 = 2 \sum_{t=1}^{n} y_t \sin(w_j t)/n,
\]
Periodogram of 25OHD serum concentrations and highest frequency

0.083 Highest frequency: Annual cycle (12 months)

Evaluated at the natural frequencies

Sine and Cosine functions

- The **steady rise-and-fall** of the cosine and sine functions makes them ideal for modeling seasonality or circadian patterns.

- This rise-and-fall pattern is **repeat**.
Sine and Cosine functions

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- This rise-and-fall pattern is **repeat**.
- This repeating property of the sine and cosine functions means that we only need to consider times from $0$ to $\leq 2\pi$. 

Circular Time

The value of $2\pi$ is a key constant because it is the circumference of a circle with radius 1.
**Sine and Cosine functions**

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Circular Time

The value of $2\pi$ is a key constant because it is the circumference of circle with radius 1.
Sine and Cosine Functions

One cycle per $2\pi$ units of time

Two cycles per $4\pi$ units of time
Sine and Cosine Functions

One cycle per $2\pi$ units of time

Two cycles per $4\pi$ units of time

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Results: assessing periodicity

Kernel smoother and Fourier Transformation

- We used a non-parametric **EPANECHNIKOV** Kernel density estimation to estimate the **probability density function** of PPROM and PA cases over time (24 hours).

- We used an **OPTIMAL** smoothing parameter because its density estimate is close to the true density.
Results: assessing periodicity

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Sensitivity analysis: cosinor vs. the best good need of fit.

Restricted Cubic spline function with $K$ knots

\[
f(x) = \sum_{j=0}^{3} \beta_0 x^j + \sum_{k=1}^{k} \beta_k (x - t_k)^3
\]

\[y_i = f(x_i) + \epsilon_i\]

Different number of Knots were placed at regularly spaced intervals (two hours, four and six hours).

Polynomial trigonometric regression

\[Y_i = \beta_0 + \beta_1 \sin(w_t) + \beta_2 \cos(w_t) + \beta_3 \sin^2(w_t) + \beta_4 \cos^2(w_t) + (...) + \beta_d x^d + \epsilon_i\]

The number of trigonometric terms in the polynomial regression was selected using a k-fold cross validation strategy.
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**Restricted Cubic spline function with** $K$ **knots**

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Moderate PPROM 32 to 36 gestation weeks

NCCP MODERATE PPROM CASES (32 to 36 gestation weeks)
Moderate Preterm Premature Rupture of Membranes NCPP, n= 1736

NCPP spontaneous singleton PPROM cases, (N=1736, 32 to 36 gestation weeks)

Circadian Test p-value <0.01

©MA Luque-Fernandez et al., non published data. Under review
NCCP EXTREMELY AND VERY PPROM CASES (<32 gestation weeks)
Extremely and Very Preterm Premature Rupture of Membranes NCPP, n= 526

NCPP PPROM spontaneous singleton cases, (N= 526, <32 gestation weeks)

Circadian Test p-value >0.05

©MA Luque-Fernandez et al., non published data. Under review
MODERATE PRETERM PA 32 to 36 gestation weeks

NCCP MODERATE PRETERM PA cases
Moderate Preterm Placental Abruption NCPP, n = 83

NCPP Preterm spontaneous singleton PA cases, (N=83, 32 to 36 gestation weeks)

Circadian Test p-value >0.05

© MA Luque-Fernandez et al., non published data. Under review
NCCP EXTREMELY AND VERY PRETERM PA cases
Extremely and Very Placental Abruption cases, NCPP, n= 60

NCPP spontaneous singleton PA cases, (N=60, <32 gestation weeks)

Circadian Test p-value >0.05

©MA Luque-Fernandez et al., non published data. Under review