RESEARCH ARTICLE

Correlation of cervical progesterone levels to plasma progesterone levels in normal pregnancy and preterm labor: A cross-sectional study [version 1; peer review: 2 approved with reservations]

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V1 First published: 15 Feb 2019, 8:189
<https://doi.org/10.12688/f1000research.17525.1>

Latest published: 25 Oct 2022, 8:189
<https://doi.org/10.12688/f1000research.17525.2>

Abstract

Background: Theory of “functional progesterone withdrawal” explains the role of progesterone prior to delivery. Previous studies mentioned the existence of progesterone regulation in the cervix that plays a role in maintaining the integrity of the cervix and cervical ripening. Cervical progesterone levels relate to activities of progesterone at the cervix, compared to its amount in circulation. The objective of this study was to measure cervical mucus progesterone levels and its correlation to plasma progesterone levels in pregnancy.

Methods: This was a cross sectional study conducted in January-September 2010 at Persahabatan Hospital. The subjects were pregnant woman in the 28th – 34th weeks of gestational age. In total, 72 subjects who met the criteria were divided into normal pregnancy group and preterm labor group. The cervical and plasma progesterone levels were measured using The Advia Centaur® Progesterone kit, which is a commercial immunoassay with direct chemiluminescence method.

Results: There was positive correlation ($r=0.539$) between cervical progesterone levels with plasma progesterone levels in the preterm labor group. There was no correlation between cervical progesterone levels with plasma progesterone levels in the normal pregnancy group.

Conclusion: This study showed that cervical progesterone levels could be measured through cervical mucus. A significant positive correlation was found by this study between cervical progesterone levels and plasma progesterone levels in the preterm labor group.

Open Peer Review

Approval Status ? ?

	1	2
version 2 (revision) 25 Oct 2022		
version 1 15 Feb 2019	? view	? view

1. **Pei F. Lai** , Imperial College London, London, UK

2. **Charles Bitamazire Businge**, Walter Sisulu University, Mthatha, South Africa

Any reports and responses or comments on the article can be found at the end of the article.

This study is expected to provide new insights for understanding the metabolism and the role of progesterone in maintaining cervical integrity during pregnancy, and its relation to prevention of preterm birth.

Keywords

Cervical, Progesteron

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Author roles: **Lisnawati Y:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Validation, Writing – Original Draft Preparation; **Wibowo N:** Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Validation, Writing – Review & Editing; **Mariana A:** Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work is supported by Hibah PITTA 2018 funded by DRPM Universitas Indonesia No.5000/UN2.R3.1/HKP.05.00/2018

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Lisnawati Y, Wibowo N and Mariana A. **Correlation of cervical progesterone levels to plasma progesterone levels in normal pregnancy and preterm labor: A cross-sectional study [version 1; peer review: 2 approved with reservations]** F1000Research 2019, 8:189 <https://doi.org/10.12688/f1000research.17525.1>

First published: 15 Feb 2019, 8:189 <https://doi.org/10.12688/f1000research.17525.1>

Introduction

Preterm birth contributes long and short-term effects to neonatal health problems. The incidence of preterm birth has not shown a significant decline. About a decade ago, the incidence of preterm birth was reported around 12–13% in United States and 5–11% in developed countries^{1,2}. In Indonesia the rate of preterm birth is 15.5% and is included in the group of 10 countries with the highest preterm birth according to the World Health Organization 2013³.

The hormone progesterone maintains the continuity of pregnancy in order to prevent preterm labor that can cause preterm delivery. Progesterone is a steroid hormone which has important role of maintaining uterine quiescence until the pregnancy reaches term. A recent study stated that circulatory progesterone level in humans remains stable until placenta is born. Nevertheless, the role of progesterone in the onset of labor is still believed to occur through an indirect mechanism called “functional progesterone withdrawal”^{4,5}.

Circulation of progesterone hormones may enter the peripheral tissue such as salivary glands and cervix. Progesterone regulation also occurs in the uterus and cervix. The uterus produces 5 α -reductase enzyme and 20 α -hydroxysteroid dehydrogenase enzyme (20 α -HSD) which converts progesterone into inactive metabolites and decreases the uterine quiescence⁶. Mahendroo *et al.*⁶ showed that in a term rat uterus with 5 α -reductase deficiency, the uterus remains to contract although delivery does not happen because there was no cervix maturation.

Andersson *et al.*⁷ showed that during pregnancy, cervical glandular epithelial cells produce 17 β -hydroxysteroid dehydrogenase (17 β -HSD) type 2 enzyme that converts estradiol to estrone and 20 α -hydroxyprogesterone (20 α P) to progesterone. Approaching delivery, regulation of 17 β -HSD type 2 decreases and creates a state that supports cervical maturation. The data on the study by Andersson *et al.* supports the idea that cervical maturation and myometrial contraction in labor involves regulation of progesterone in the cervix and uterus through the complex relationship between cervical epithelial, stromal, and myometrium⁷.

Progesterone plasma level is widely studied to monitor the function of the corpus luteum in secretory phase and the beginning of pregnancy. In later pregnancy, progesterone level monitoring is only done for individuals with a high risk of having abortus or preterm delivery, although the effectivity of its application in clinical practice remains controversial⁸.

Assumption about the reduced quantity of plasma progesterone before delivery is now widely questioned. Currently, the role that progesterone plays before delivery is described as “progesterone functional withdrawal” theory. Prior studies mention that there is progesterone regulation in the cervix, which contributes to preserve cervical integrity as well as cervical maturation^{6,7}. It is assumed that cervical progesterone levels reflect the target organ’s progesterone activity, i.e. the cervix,

better than circulatory progesterone. Therefore, measuring cervical mucus progesterone during normal pregnancy and preterm delivery pregnancy needs to be explored. As far as we know, there has been no research that measures levels of progesterone in cervical mucus.

Methods

Study design

This study was a cross-sectional correlation study conducted in January 2010 until September 2010 at Persahabatan General Hospital Jakarta, Indonesia.

Participants

The participants are subjects in the 28th – 34th weeks of pregnancy with a single and normal fetus. The subjects were divided into two groups, normal pregnancy and preterm labor, as described in the inclusion criteria.

The inclusion criteria for this study were as follows: (1) pregnant women at 28th – 34th weeks of pregnancy with single and normal fetus who attended at Persahabatan General Hospital, (2) subjects in normal pregnancy group had no contraction, (3) subjects in preterm labor group had minimal 4 contractions in 20 minutes that was proved by cardiotocography, without amniotic membrane ruptured or blood mucus in cervix, (4) subject willing to take part in research and sign informed consent. The exclusion criteria for this study were as follows: (1) respondents who does not meet the inclusion requirements for this study; and (2) respondents who not willing to be a participant in this study and incomplete of filling informed consent.

To anticipate differences in progesterone levels every week of gestational age, subgroup analysis was carried out based on gestational age. The subjects of the study were recruited according to three subgroup of gestational age in each group of normal pregnancy and preterm labor: (1) 28 (+0 days) up to 29 (+6 days) weeks, (2) 30 (+0 days) up to 31 (+6 days) weeks, and (3) 32 (+0 days) to 33 (+6 days) weeks.

The sample size was derived from the formula $n = \left(\frac{Z_{\alpha/2} + Z_{\beta}}{r \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \right)^2 + 1$, a sample of at least 12 subjects in each subgroup is needed to detect a moderate correlation ($r = 0.75$) with 80% power and 5% confidence limit, calculated using the hypothesis test formula for Pearson correlation. Based on this calculation, we enrolled 72 participants, 36 subjects in the normal pregnancy group and 36 subjects in the preterm labor group.

Data collection

In both group participants, they were given an explanation of the purpose and benefits of the study, and as asked to provide written consent to participate in the study. The written approval sheet was signed by the patient and the researcher. After the patients signed the written approval sheet and also have given the information by the researcher, maternal blood (5 mL) was collected from patients to measure progesterone and cervical plasma. The blood samples were centrifuged on 3000 rpm for 15 minutes to obtain the plasma/serum.

Cervical mucus was collected using prism shaped MQA ophthalmic sponge. The speculum was inserted inside the vagina on lithotomy position to visualize the cervix with enough lighting to inspect. The ophthalmic sponge was hold on the base of the prism using long forceps/tweezers and collection of cervical mucus was performed by inserting the ophthalmic sponge into the external orifice of the uterus and hold still for 60 seconds until the sponge absorbs the mucus. The sponge was removed gently and put inside a storage tube, a neutral vacuum tube filled with 1 cc of 0.9% NaCl to store the specimen. The tube was put inside a cooler (2–8°C) within 30 minutes of collection to prevent contamination and the tube was put inside a freezer within 4 hours after collection. Before processing, samples were centrifuged at 16000 g to obtain the supernatant (to separate sample from sponge)⁹.

The plasma blood and cervical mucus progesterone levels were measured using The Advia Centaur® Progesterone kit, which is a commercial immunoassay with direct chemiluminescence method. This kit has 0.21 µg/L (0.67 nmol/L) analytical sensitivity. The score used in the analysis were between 0.21 µg/L to 60.00 µg/L.

Statistical analysis

After all the necessary data was collected, the data was coded on pre-arranged coding sheets by the principal investigator. Statistical analyses and data entry were performed using the Statistical Package for the Social Sciences (SPSS), version 20.0. We performed statistical analysis using a Spearman test for measures the correlation or the strength of association between cervical progesterone level and plasma progesterone level in normal pregnancy group and the preterm labor group. The results were presented in tables and figures.

Ethical considerations

This study was approved by The Ethics Committee of Persahabatan General Hospital (approval number 01/Diklit-RSP/

Kom.Etik/II/2010). The research proposal was submitted and reviewed by the Research Committee, whereby permission was granted to conduct the research. A written letter of consent was submitted to the health institution to seek permission to conduct this study, which was also granted. Privacy and confidentiality of the clients' information was observed through the use of data collection with coded identification numbers. All prospective subjects received an explanation from the main researcher and additional researchers regarding the procedures for conducting research. The decision to follow or refuse to follow the research was taken by informed consent. All data will be kept confidential and the subject had the right to know all the results of the examination carried out.

Results

This study enrolled a total of 72 pregnant women who met the study criteria. They were divided into two groups: 36 women in the normal pregnancy group and 36 women in the preterm labor group. **Table 1** shows that there were no significant differences in age distribution, education level and working status between groups. Gestational age grouping was balanced evenly between the two groups.

Data distribution of cervical progesterone levels in normal pregnancy group and preterm labor group were not normal due to extreme values. **Table 2** shows that the median of the cervical progesterone level of normal pregnancy group was 1.74 ng/ml, which is lower than preterm labor group (median: 1.91 ng/ml). The range of progesterone levels in the preterm labor group was greater than in normal group. Plasma progesterone levels in both groups had normal data distribution. Mean value of normal pregnancy group was 174.52 ± 59.11 ng/ml, lower than preterm labor group (195.10 ± 82.21 ng/ml).

The correlation between cervical progesterone levels and plasma progesterone levels in normal pregnancy group were show using a scatter plot graph (**Figure 1**). Although schematically there was

Table 1. Demographic characteristics.

Variables		Group		P
		Normal	Preterm	
Age (years)				
Mean (SD)		32 (6)	29 (7)	0.107*
Gestational age (week)	28-30	12 (50)	12 (50)	1.000
n (%)	30-32	12 (50)	12 (50)	
	32-34	12 (50)	12 (50)	
Education Level	High School	26 (72.2)	29 (80.6)	0.405
n (%)	University	10 (27.8)	7 (19.4)	
Work	Yes	14 (38.9)	10 (27.8)	0.317
n (%)	No	22 (61.1)	26 (72.2)	

Chi Square test; Unpaired T-test*

Table 2. Cervical and plasma progesterone levels of normal pregnancy and preterm labor.

Variables	Group	
	Normal	Preterm Labor
Cervical progesterone (ng/mL)		
Med (min-max)	1.74 (0.99-5.74)	1.91 (0.37-13.72)
Plasma progesterone (ng/mL)		
Med (min-max)	164.32 (81.10-294.89)	170.23 (59.65-390.19)

Figure 1. Correlation between cervical progesterone level and plasma progesterone level in normal pregnancy group. Spearman correlation $r = 0.196$; p value = 0.251.

a trend of increasing relationship between cervical and plasma progesterone levels, the statistical test proved that there was no correlation between them ($p=0.251$; $r=0.196$). The correlation between cervical progesterone levels and plasma progesterone levels was more visible in the preterm labor group compared to the normal group (Figure 2). The test showed a significant correlation with moderate strength ($p=0.001$; $r=0.539$). This result indicated that elevated level of cervical progesterone was directly proportional to plasma progesterone level.

Discussion

As a pregnancy hormone, progesterone is not only metabolized in circulation, but also in the uterus. Previous studies have proven the existence of local regulation of progesterone in uterus and cervix. A term uterus produces an enzyme that convert progesterone into inactive metabolites and decreases the uterine quiescence^{6,7}. In this study, the cervical progesterone level could be measured through the cervical mucus, which was collected using a prism shaped ophthalmic sponge. The data obtained from both groups had abnormal distribution. The range was quite

large, especially in the preterm labor group with a median of 1.91 ng/ml (0.37–13.72). The existence of extreme values affected the distribution of data in both groups and caused abnormal data distribution. Some of the possible causes of this abnormal data distribution were bias, which could occur in collecting and processing samples; and examination methods, which still require correction and presence of cervical local factors (such as clinical or subclinical infection) that may affect the regulation of progesterone (which was not explored in this study).

Progesterone is a hormone that plays a dominant role to keep the uterus quiescence during pregnancy. Circulatory progesterone level in humans remains elevated until birth, which refers to the notion of a “functional progesterone withdrawal”, which occurs before delivery^{4,5}. The definition may also apply to cervical progesterone. Progesterone is inactivated by local regulation of the cervix, not by the quantity of the progesterone hormone. The study by Andersson *et al.*⁷ was one study that proved the existence of progesterone inactivation in the uterus and

Figure 2. Correlation between cervical progesterone levels and plasma progesterone levels in preterm labor pregnancy group. Spearman correlation $r = 0.539$; $p = 0.001$

cervix before delivery. Using immunohistochemical methods and quantitative real-time PCR, this study proved the existence of elevation of 20α -hydroxyprogesterone (20α P), an inactive metabolite of progesterone, just before delivery. This elevation was allegedly caused by decreasing concentrations of 17β -HSD type 2 enzyme in endocervical epithelial cells⁷. The method that we use was different from the methods above. We tried to measure progesterone level from cervical mucus by using competitive immunoassay with direct chemiluminescence method. This may be a limitation of our study.

In the present study, cervical progesterone levels in the normal group were lower than the preterm labor group. These results were inversely related to the expected hypothesis where cervical progesterone levels in preterm labor group were expected to be lower than the normal pregnancy group. The hypothesis, which was based on the assumption that the cervical mucus was similar with saliva and the study conducted by Connor *et al.*¹⁰ in which it was stated that salivary progesterone concentrations between 24 and 34 weeks of pregnancy were lower in pregnant women who experienced delivery before 34 weeks of gestation compared to pregnant women who experienced delivery after 37 weeks, were not proven in this study. The salivary progesterone levels in the study by Connor *et al.* were measured serially, while in this study progesterone level were measured one-time. Therefore, to get better data, serial measurements of cervical progesterone level using a good method in the pregnancy group might be needed.

High cervical progesterone levels in the preterm labor group in the present study might be caused by an increase of progesterone metabolites in the cervix due to infection process. Infection rates contributed more than 30% as a cause of preterm labor.

Mahendroo *et al.*⁶ showed conversion of progesterone into inactive metabolites by cervical enzymes before delivery in pregnant rats. Using radioimmunoassay examination technique (RIA), this study detected conversion of progesterone into 5 α -pregnan-3, 20-dione by steroid 5 α -reductase, into 4-pregnen-20 α -ol-3-one by 20 α -hydroxysteroid dehydrogenase and into 5 α -pregnan-3 α , 20 α -diol by the combined action of two enzymes with 3 α -hydroxysteroid dehydrogenase. This present study used a competitive immunoassay technique with direct chemiluminescence method which did not differentiate active progesterone to its inactive metabolite produced in the cervix. Detected progesterone level in cervical mucus possibly includes progesterone and its inactive metabolites and therefore did not reflect the actual activity of cervical progesterone.

In the normal pregnancy group, although schematically there was a trend of increasing relationship between cervical and plasma progesterone level, the result generated by the correlation test showed that there was no significant correlation with weak strength. Thus, the elevation of plasma progesterone levels was not followed by elevation in plasma progesterone level. Different results were obtained in the preterm labor group: the relationship between cervical progesterone and plasma progesterone were more visible and the result obtained from the test showed a significant relationship as well as moderate correlation strength ($r=0.539$). This result indicated that the elevation of cervical progesterone level in the preterm labor group was directly proportional to plasma progesterone levels. Differences occurred in both groups require further study. Was it influenced by the regulation of local progesterone that occurs in the cervix? As known from previous studies, the regulation of cervical progesterone during pregnancy was different than before delivery. Would inflammation/infection in the cervix influence cervical and

plasma progesterone level? A one-time sample collection was also thought to be possible factor influencing different results of both groups.

Conclusion

Cervical progesterone levels can be measured through the cervical mucus. A significant positive correlation was only found between cervical progesterone level with plasma progesterone level in the preterm labor group. This study is expected to provide new insights for understanding the metabolism and the role of progesterone in maintaining cervical integrity during pregnancy, and its relation to prevention of preterm birth.

Data availability

Underlying data

Open Science Framework: Correlation of cervical progesterone levels to plasma progesterone levels in normal pregnancy and preterm labor: A cross-sectional study, <https://doi.org/10.17605/OSF.IO/YDM9P>¹¹.

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Grant information

This work is supported by Hibah PITTA 2018 funded by DRPM Universitas Indonesia No.5000/UN2.R3.1/HKP.05.00/2018.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

This work was performed at the Department of Obstetrics and Gynecology, Persahabatan General Hospital Jakarta, Indonesia. The authors wish to thank all staff at the Persahabatan General Hospital Jakarta for supporting us regarding this study.

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 21 March 2022

<https://doi.org/10.5256/f1000research.19166.r127213>

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Charles Bitamazire Businge

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The authors need to clearly state their hypothesis or study aims. They simply stated that measuring cervical mucus progesterone during normal pregnancy and preterm delivery pregnancy needs to be explored.

In paragraph six of the introduction, they noted that *"It is assumed that cervical progesterone levels reflect the target organ's progesterone activity, i.e., the cervix, better than circulatory progesterone"*. Hence there seems to be an implied hypothesis that cervical progesterone among women with preterm labour will be lower than that of controls with normal pregnancy. However, the title of their research and the statistical approach used to calculate the sample size do not match with this implied aim/hypothesis. In the discussion, they did not put much emphasis on the correlation between serum and cervical progesterone (as the title suggests) but dwelt more on the difference between the measured levels of cervical progesterone of women with and without preterm birth. This mismatch needs to be addressed.

Also, in the introduction, the authors rightly observed that *"Assumption about the reduced quantity of plasma progesterone before delivery is now widely questioned. Currently, the role that progesterone plays before delivery is described as "progesterone functional withdrawal" theory."* If their aim was to confirm or refute the hypothesis that it is the absolute reduction of progesterone in cervical stroma instead of 'functional progesterone withdrawal' that is crucial in the process of preterm labour, this needs to be clearly stated. In this case, they will use the Mann-Whitney U test to compare the serum progesterone levels and the cervical progesterone levels of the two groups (since the data is not normally distributed). They can then correlate the cervical and serum levels of each group as a secondary aim. This can then be followed by an appropriate discussion of the results as they relate to the aim of the study.

The unexpectedly higher levels of serum and cervical progesterone observed by the authors may be due to chance alone given the small sample size. Several related studies have reported that low serum progesterone is associated with preterm labour (Ku *et al.*, 2018¹; Pratama *et al.*, 2020²).

Despite having high serum and cervical progesterone, some women may be at increased risk of preterm labour due to increased metabolism of progesterone in the target tissues (Patil *et al.*, 2020³) or other mechanisms.

The authors concluded that their study was expected to provide new insights for understanding the metabolism and the role of progesterone in maintaining cervical integrity during pregnancy, and its relation to the prevention of preterm birth. However, there is no data from their study to support this statement.

Several sentences require editing so as to improve on clarity.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Obstetrics and Gynaecology, public health, micronutrients and cardiovascular disease

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Sep 2022

Yuyun Lisnawati, Persahabatan General Hospital, Jakarta, Indonesia

1. The aim of the study and the hypothesis were added to the study.
2. We thank you for the comment toward our manuscript. We are also aware to the problem and made the changes accordingly
3. The additional statistical test was performed in our study. However, there were no significant difference of both cervical and serum progesterone between study groups. We have also added the discussion to our discussion part.
- 4-5. Added to the respective section.
6. We are also aware to the issue. The statement has been omitted.
7. Various grammatical errors and confusing sentences were altered accordingly.

We hope that our response will sufficiently address your concern toward our manuscript. If you have any other comment or recommendation, please let us know. We thank you once again for your cooperation.

Sincerely yours,

Yuyun Lisnawati

Competing Interests: There was no competing interests to be disclosed.

Reviewer Report 28 July 2021

<https://doi.org/10.5256/f1000research.19166.r89602>

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Pei F. Lai

Division of Reproductive and Developmental Biology, Imperial College London, London, UK

In this article, the authors highlight that cervical progesterone action during human preterm labour is best characterised by profiling the local (rather systemic) availability of this steroid in pregnant women, which is attributed to the concept of functional progesterone withdrawal. Their

aim was to determine whether the correlation in progesterone concentration between maternal peripheral blood plasma and cervical mucus is different between non-labouring and preterm labouring women, when analysed at 28-34 weeks of gestation.

The effort made to obtain preterm gestation-matched samples for comparison of non-labouring and labouring women was good. However, the authors need to present more measurements with statistical analyses for their sample groups to sufficiently establish the extent to which local (cervical) progesterone is more important than circulating (blood) progesterone in the manifestation of preterm labour. The relevance of their findings to the clinical efficacy of progesterone prophylaxis for preterm labour prevention (evaluated by several published clinical studies, refer to Romero *et al.*, 2016¹ and EPPPIC Group, 2021²) also requires more detailed consideration to improve the article.

Additionally, comments for each section of the article are as follows:

Introduction:

- Localised regulation of progesterone metabolism is only one possibility for how functional progesterone withdrawal is mediated. The other (relatively more explored) possibility involves changes in tissue progesterone receptor expression/function irrespective of ligand concentration; this should be acknowledged with the inclusion of relevant references, though only needs to be mentioned briefly because it was not the focus of the authors' study.
- It would be helpful to explicitly state the hypothesis of the study in the Introduction (rather than give it first mention in the Discussion).

Methods:

- For the participant inclusion/exclusion criteria, please state whether tocolytics or steroids were administered prior to sample collection. Please also describe the method used to confirm absence of fetal membrane rupture.
- Further detail should be added to Table 1 to make the data more valuable for potential future integration with similar datasets from other sources. For example, gravida, parity, ethnicity, body mass index, history of preterm birth, type of preterm labour (e.g. idiopathic, placental abruption, chorioamnionitis), Bishop score at time of sampling, gestation at fetal delivery, and mode of fetal delivery.
- Please include a citation for the power calculation formula, to explain what each factor in the calculation represents (to help with interpretation by those who are unfamiliar with this specific formula).
- The "300 rpm" centrifugation speed (in first paragraph of the 'Data collection' section) needs to be converted to standard relative centrifugal force (g unit).
- Please state whether the blood serum or cervical mucus samples needed to be diluted prior to use at the immunoassay for quantifying their progesterone concentrations; also include mention of any control samples that were used to evaluate assay accuracy.

Results:

- It is stated in the Methods section that “To anticipate differences in progesterone levels every week of gestational age, subgroup analysis was carried out based on gestational age” – this subgroup consideration is not shown in the data presented, despite it being a good approach. The authors need to either include their findings from this subgroup analysis or explain why they decided not to proceed with it.
- Figures 1 and 2 show that progesterone concentrations, in both blood plasma and cervical mucus, for the two participant groups of interest were within very similar ranges to each other; this is in agreement with median values presented in Table 2. Can the authors comment on whether the wider min-max range for cervical mucus samples from the preterm labour group (shown in Table 2) was mostly attributed to the six samples that contained >6 ng/mL progesterone (shown in Figure 2)? Was there anything clinically distinct about these six women, when compared to the other 30 women in the preterm labour group?

Discussion & Conclusion:

- Possible explanations for abnormal data distribution for both participant groups are good. Can the authors add further comment with regards to possibility of infection for both groups of participants, especially whether it was a consideration for their inclusion/exclusion criteria for participant selection?
- Progesterone concentration in blood plasma from preterm pregnant women has been quantified in previous studies (e.g. Smith *et al.*, 2009³). The authors should add depth to their discussion by commenting on how comparable their dataset is to these previous studies; with consideration of any differences in participant demographics, clinical observations and sampling methodology.
- In the third paragraph, the authors should be more cautious about their interpretation of differences in cervical progesterone concentration between their preterm labour and normal pregnancy groups, especially because they did not present statistical analysis outcome for the corresponding data (Table 2) that shows notable overlap of min-max ranges.
- The authors should provide any technical information regarding cross-reactivity of the immunoassay (in the Methods section), which would help to judge to what extent we should be concerned about “Detected progesterone level in cervical mucus possibly includes progesterone and its inactive metabolites and therefore did not reflect the actual activity of cervical progesterone” (fourth paragraph). Additional assays (ideally using either a different immunoassay kit or methodology) with control samples (of metabolites) should have been undertaken to ascertain accuracy of progesterone quantification, where high cross-reactivity with its metabolites was likely to occur.
- The following sentence needs correcting: “Thus, the elevation of plasma progesterone levels was not followed by elevation in plasma progesterone level” (fifth paragraph).
- The last sentence of the Conclusion is an overstatement until more observations and findings are added to the article. If samples are still available for use, the authors should consider acquiring data for quantification of specific progesterone metabolites (such as those identified in references 6 and 7) to assess whether preterm labour is associated with

changes in their concentrations more at the cervix than maternal blood.

References

1. Romero R, Nicolaides KH, Conde-Agudelo A, O'Brien JM, et al.: Vaginal progesterone decreases preterm birth ≤ 34 weeks of gestation in women with a singleton pregnancy and a short cervix: an updated meta-analysis including data from the OPPTIMUM study. *Ultrasound Obstet Gynecol.* 2016; **48** (3): 308-17 [PubMed Abstract](#) | [Publisher Full Text](#)
2. EPPPIC Group: Evaluating Progestogens for Preventing Preterm birth International Collaborative (EPPPIC): meta-analysis of individual participant data from randomised controlled trials. *Lancet.* 2021; **397** (10280): 1183-1194 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Smith R, Smith JI, Shen X, Engel PJ, et al.: Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. *J Clin Endocrinol Metab.* 2009; **94** (6): 2066-74 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Progesterone and cAMP signalling in human myometrium during pregnancy and labour, with specific interest in preterm labour.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 Sep 2022

Yuyun Lisnawati, Persahabatan General Hospital, Jakarta, Indonesia

1. We thank you for the comment and addition to our manuscript. We were also aware to

the issues presented. We have added a comparison of both cervical and serum progesterone level between normal pregnancy and preterm labor. However, the results were not significant. We have also added the possible reason and the prior studies about the issue.

2. Added to the introduction section

3. The aim of the study and the hypothesis were added to the study.

4. In this study, the tests were performed prior to the tocolytics or steroid administration to ensure that those would not become confounding factors. Furthermore, the absence of fetal membrane rupture was proved by ultrasound examination. Added to the inclusion / exclusion criteria.

5. We agree to the comment. However, we are very sorry to tell you that the details to our demographic characteristics is currently very limited. Therefore, it cannot be added to Table 1.

6. Our calculation was based on the assumption that a moderate correlation would have moderate correlation with serum progesterone. We have changed the section accordingly.

7-8. Revised accordingly.

9. We are very sorry for the mistake in our wording. The sentence has been omitted as the correct process was the normal pregnancy group subjects were matched for age and gestational age to the preterm labor group.

10. We have also observed the phenomenon in our previous discussion. However, there were no clinically significant differences between the subjects and other subjects.

11. Added to the discussion section, especially on the infection being one of the confounding factors in this study.

12-13. Added to the respective section.

14. We are aware of the issue. However, there was no additional examination performed with the same sample. Therefore, we can only explain the issue as a limitation to our study and it should be performed in future studies.

15. Omitted.

16. We agree that the later part of our conclusion is an overstatement. Therefore, it was omitted

Competing Interests: The authors stated that there was no conflict interest to be disclosed

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