Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

Original Research

The Effect of Establishing *Escherichia coli* on Balb/C Rats as A Model of Preterm Labor

Suheni Ninik Hariyati[1]*, Dewi Santosaningsih[2], Arief Satria Hardika[3], Fikhy Rizky Hapsari[1], Nirwan Nugraha L.[1], Wildan Aulia Firdaus[1]

[1] Department of Obstetrics and Gynaecology, Faculty of Medicine, Universitas Brawijaya/ Saiful Anwar General Hospital, Malang, East Java, Indonesia
[2] Department of Clinical Microbiology, Faculty of Medicine, University of Brawijaya/ Saiful Anwar General Hospital, East Java, Indonesia
[3] Department of Anatomical Pathology, Faculty of Medicine, University of Brawijaya/ Saiful Anwar General Hospital, East Java, Indonesia

ARTICLE HISTORY
Received: 2 July 2023
Revised: 18 July 2023
Accepted: 25 August 2023

CORRESPONDING AUTHOR*
Suheni Ninik Hariyati
heni_ota@yahoo.com
Department of Obstetrics and Gynaecology, Faculty of Medicine, Universitas Brawijaya/ Saiful Anwar General Hospital, Malang, East Java, Indonesia

KEYWORD
Escherichia coli; Preterm labor; Balb/C mice

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

ABSTRACT

Introduction: The prevalence of preterm labor in Indonesia is estimated at 7-14%, which is around 459,200-900,000 babies per year. The mechanism of infection is the biggest cause of preterm labor. The bacteria that often causes infection is *Escherichia coli*. This study aims to see the effect of giving *Escherichia coli* to produce premature models of mice.

Material and Methods: This study used a true experimental method with a post-test only control group design approach. The independent variables were preterm labor & cervical tissue culture, amniotic membranes, and placenta. The dependent variable of the study was the administration of *Escherichia coli* 10^9 CFU to the cervix. This research will be tested with a comparative test.

Results: The treatment group were given *Escherichia coli* experienced parturition with the fastest time of 38 hours 22 minutes and the longest time of 42 hours 12 minutes. *Escherichia coli* colonies on Eosin Methylene Blue (EMB) culture media with a glowing green image was obtained. Cervical culture, amniotic membranes and placenta from the treatment group of Balb/C mice showed the growth of *Escherichia coli* colonies. Neutrophil infiltration and edema in the cervical stroma was found to be more severe in Balb/C mice that received treatment with *Escherichia coli* 10^9 CFU/ml.

Conclusion: *Escherichia coli* shortens the time of delivery and increases the bacterial load in pregnant Balb/C mice.

Cite this as: Hariyati SN, Santosaningsih D, Hardika AS, Hapsari FR, Nugraha N, Firdaus WA. (2023) The Effect of Establishing *Escherichia coli* on Balb/C Rats as A Model of Preterm Labor. *Asian J Heal Res.* 2 (2): 50–55. doi: https://doi.org/10.55561/ajhr.v2i2.115

INTRODUCTION

Premature delivery can lead to complications. Maternal complications such as increased risk of postpartum hemorrhage and infection. While neonatal complications can cause morbidity and mortality in infants. Therefore, the prediction and prevention of preterm labor is one of the main priorities in health services [1–5]. In 2018, WHO noted that there were 675,700 premature deliveries in Indonesia, there has been a decline in the number of preterm deliveries, the decline tends to slow down in the last 10 years. The prevalence of preterm labor in Indonesia is estimated at 7-14%, which is around 459,200-900,000 babies per year [6–8].

The mechanism of infection is the biggest cause of preterm labor, which is about 25-40% and ascending vaginal infection is the most common route of infection. The bacteria that often causes infection is *Escherichia coli* [9,10]. In preterm labor, infection/ inflammation (chorioamnionitis and uteroplacental insufficiency) causes changes in the architecture of the collagen fibrils resulting in rupture of the amniotic membrane and remodeling of the cervix which is necessary for labor [11–14].

This study aims to see the effect of giving *Escherichia coli* to the cervix on preterm labor so that it can produce premature models of mice which can later be used as research models for premature labor.

MATERIAL AND METHODS

Study Design

This study used a true experimental method with a post-test only control group design approach which was carried out from September to October 2021. This study consisted of 2 stages, firstly determining the dose of *Escherichia coli* used in Balb/C mice to produce preterm labor and then evaluating the average parturition of Balb/C mice after being given treatment. Balb/C pregnant mice were obtained from Laboratorium Penelitian dan Hewan Coba (LPHC) Fakultas Kedokteran Universitas Brawijaya Malang and *Escherichia coli* cultures are made in the microbiology laboratory of Fakultas Kedokteran Universitas Brawijaya Malang, while the histopathological calculation of *Escherichia coli* was carried out in the Anatomical Pathology Laboratory. Inclusion criteria were male and female Balb/C mice aged 2 months, average weight 20-25 grams, healthy condition characterized by active movement, bright and unblemished eyes, Balb/C mice with their first pregnancy. The exclusion criteria were previously for other experiments. The independent variables were preterm labor & cervical tissue culture, amniotic membranes, and placenta. The dependent variable of the study was the administration of *Escherichia coli* 10^9 CFU to the cervix. This research will be tested with a comparative test.

Data Analysis

From this treatment it was proven that giving Escherichia coli 109 CFU/ml caused pregnant Balb/C mice to experience premature parturition. It was proven by examining the microbiological culture of Escherichia coli on Eosin Methylene Blue media, then calculating the bacterial load and histopathological examination of anatomical pathology to see neutrophil infiltration in the cervical epithelium and an appearance of edema in the cervical stroma.

RESULTS

Based on Table 1, 32 pregnant Balb/C mice were divided into 2 groups, namely 16 control group and 16 treatment group. Based on the observation of the control group of pregnant Balb/C mice, 16 mices experienced labor over 19 days, with the fastest time is 19 days 20 hours and the longest time is 19 days 30 hours (Table 2).

While the treatment group of pregnant Balb/C mice on the 15th day were given *Escherichia coli* 10^9 CFU/ ml on the cervix, 16 mices experienced parturition with the

Table 1. Observation of the Control Group of Pregnant Balb/C Mice

Number of Mice	Day 19th	Time Lapes
1	October 21th, 2021	26 hours 20 minutes
2	October 21th, 2021	27 hours 5 minutes
3	October 21th, 2021	20 hours 55 minutes
4	October 21th, 2021	20 hours 40 minutes
5	October 21th, 2021	28 hours 3 minutes
6	October 21th, 2021	27 hours 5 minutes
7	October 21th, 2021	30 hours 8 minutes
8	October 21th, 2021	24 hours 10 minutes
9	October 21th, 2021	25 hours 4 minutes
10	October 21th, 2021	26 hours 8 minutes
11	October 21th, 2021	24 hours 10 minutes
12	October 21th, 2021	25 hours 5 minutes
13	October 21th, 2021	23 hours 7 minutes
14	October 21th, 2021	22 hours 35 minutes
15	October 21th, 2021	26 hours 12 minutes
16	October 21th, 2021	25 hours 16 minutes

fastest time of 38 hours 22 minutes and the longest time of 42 hours 12 minutes. In the control group, *Escherichia coli* colony growth was not found on Eosin Methylene Blue (EMB) culture media, so that bacterial load was not calculated. In the treatment group, after it was proven that the growth of *Escherichia coli* colonies on Eosin Methylene Blue (EMB) culture media with a glowing green image was obtained, then the number of colonies that grew (Bacterial load) was calculated (Fig.1).

The results of cervical culture, amniotic membranes and placenta from the treatment group of Balb/C mice showed the growth of *Escherichia coli* colonies. In normal parturition, there is a picture of neutrophil infiltration in the cervical epithelium and edema in the cervical stroma. The description of neutrophil infiltration and edema in the cervical stroma was found to be more severe in Balb/C mice that received treatment with *Escherichia coli* 10^9 CFU/ml on the cervix which indicated inflammation (Table 3).

Table 2. Observation of Treatment Group of Pregnant Balb/C Mice

Number of Mice	Giving *Escherichia coli*	Parturition Time	Time Lapse
1	16.00	07.05	39 hours 5 minutes
2	16.02	08.00	39 hours 58 minutes
3	16.04	10.15	42 hours 11 minutes
4	16.06	09.10	39 hours 4 minutes
5	16.08	06.30	38 hours 22 minutes
6	16.10	07.10	39 hours
7	16.12	08.15	40 hours 3 minutes
8	16.14	08.20	40 hours 6 minutes
9	16.16	09.20	41 hours 4 minutes
10	16.18	10.30	42 hours 12 minutes
11	16.25	08.25	40 hours
12	16.27	07.45	39 hours 18 minutes
13	16.29	07.50	39 hours 21 minutes
14	16.31	08.10	39 hours 39 minutes
15	16.33	07.35	39 hours 2 minutes
16	16.35	09.55	41 hours 20 minutes

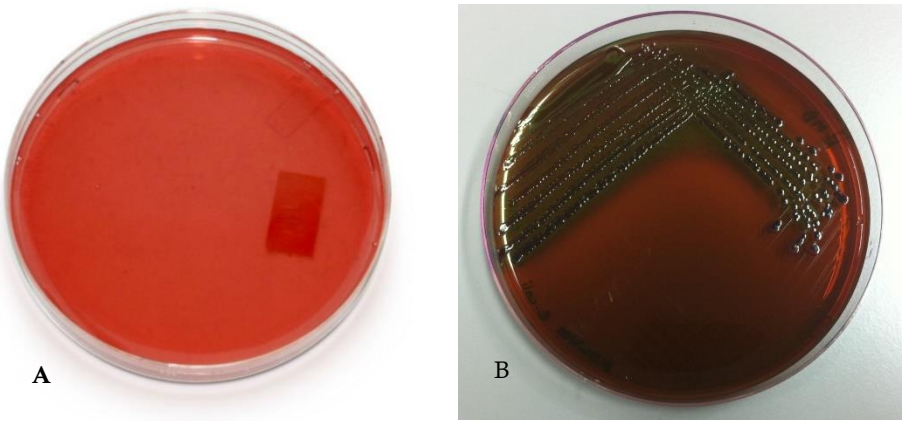


Fig.1. The Results of Cervical, Amniotic Membranes and Placenta Culture. Colony growth was not found on Eosin Methylene Blue in the control group (A); culture media with a glowing green image was obtained in treatment group (B)

Table 3. The Results of Cervical Culture, Amniotic Membranes and Placenta from The Treatment Group of Balb/C Mice

Number of Mice	Cervical	Amniotic Membrane	Placenta
1	4087607,14	960766,70	90083,57
2	2543400,00	754870,00	15670,40
3	1544207,14	690876,67	56732,00
4	2089221,43	272507,14	199838,57
5	1635042,86	164907,32	76807,07
6	2815100,00	327098,50	97198,50
7	3451757,14	372654,82	96258,65
8	2270892,86	325790,86	106277,00
9	2089221,43	253987,07	108170,70
10	1725878,57	353890,00	82660,00
11	3541785,71	231980,00	55409,00
12	2452564,29	254309,00	63585,00
13	1725878,57	175098,00	65894,65
14	2905128,57	272507,14	158054,00
15	1362535,71	102435,85	93560,76
16	1907357,14	342980,00	165320,00

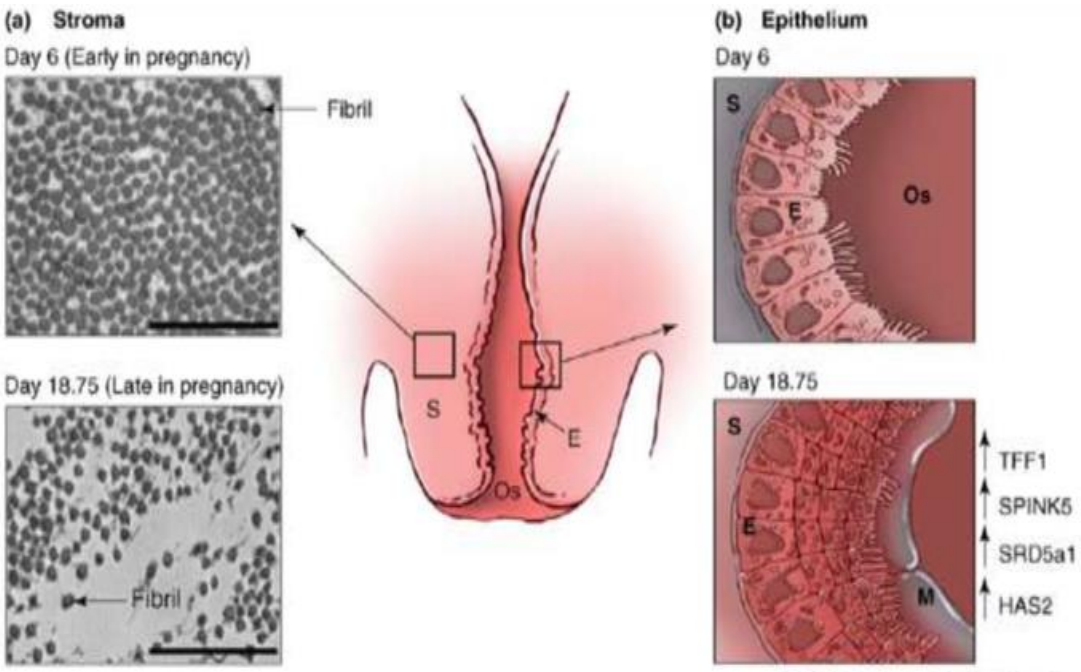


Fig.2. Features of Edema in the Cervical Stroma (A) and Neutrophil Infiltration in the Cervical Epithelium (B).

DISCUSSION

Giving *Escherichia coli* 10^9 CFU/ml to the cervix of pregnant Balb/C mice on the 15th day caused Balb/C mice to have premature labor. This is in accordance with research conducted by Ronald McCarthy et al. that showed several types of mice and rats by comparing the administration of *Escherichia coli* and the administration of LPS to induce preterm labor. One of the results is that in Balb/C mice the administration of *Escherichia coli* performed intracervical will result in 92% preterm labor at a dose of 2-10 x 10^5 CFU/ml (16). In this study, the dose of *Escherichia coli* was 10^9 CFU/ml, the difference in the dose of *Escherichia coli* given was due to the different virulence of *Escherichia coli* in accordance with the statement of Grohmann et al in 2018 that *Escherichia coli* is a genus with only one species having several hundred antigenic types. This type is characterized by different combinations. O antigen (somatic) which is heat-resistant or thermostable, consisting of lipopolysaccharide containing glucosamine and present in the cell wall of gram-negative bacteria plays a role in determining the virulence level of *Escherichia coli* [17]. This dose corresponds to the type of *Escherichia coli* in the Microbiology laboratory of Fakultas Kedokteran Universitas Brawijaya.

In accordance with the statement of Mondal et al, 2022 that good growth at an optimal temperature of 37ºC in media containing 1% peptone as a carbon and nitrogen source. *Escherichia coli* ferments lactose and produces indole which is used to identify bacteria in culture media, can survive up to 60ºC for 15 minutes or at 55ºC for 60 minutes. *Escherichia coli* grows well at temperatures between 8°- 46°C and an optimum temperature of 37°C. If bred below the minimum temperature or slightly above the maximum temperature, it will not die immediately but is in a state of dormancy. *Escherichia coli* only recognizes one type of reproduction, namely by sexual or vegetative means of rapid reproduction [18–19].

Escherichia coli infection is most commonly found in the cervix, followed by the amnion and placenta. This is in accordance with research conducted by Elovitz et al, 2004 that the pathogenesis of preterm labor caused by infection causes bacterial colonization of the vagina during pregnancy to ascend through the uterine cervix and then due to virulence or due to a deficiency of genetic or immunological responses from the mother, the bacteria then replicate in the amniotic membrane, at this point, the mother's inflammatory response will be induced, so that the bacteria continue to reproduce and enter the placenta, then enter the fetal circulation through the umbilical cord. At this stage, the fetal inflammatory response will be induced. Bacteria can also enter the amniotic cavity through the amniotic membrane [20].

The description of neutrophil infiltration and edema in the cervical stroma was found to be more severe in Balb/C mice that received treatment with *Escherichia coli* 10^9 CFU/ml on the cervix [21–22]. In accordance with the results of the bacterial load that the number of *Escherichia coli* bacteria was found to be the highest in the cervix, followed by the amnion and placenta, the anatomical pathology picture showed that inflammation was more severe in the cervix than the amnion and placenta.

CONCLUSION

Escherichia coli shortens the time of delivery and increases the bacterial load in pregnant Balb/C mice.

ACKNOWLEDGMENT

We thank anonymous referees for their valuable suggestions.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. Di Renzo GC, Tosto V, Giardina I. The biological basis and prevention of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018 Oct;52:13–22.
2. Melchor Corcóstegui I, Unibaso Rodríguez E, Ruiz Blanco N, Nikolova T, Nikolova N, Burgos San Cristóbal J, et al. Is mid-trimester cervical length screening effective for reduction of threatened preterm labor? Taiwan J Obstet Gynecol. 2023 May;62(3):412–6.
3. Panada Sedianing Drastita, Hardianto G, Fitriana F, Utomo MT. Faktor Risiko Terjadinya Persalinan Prematur. Oksitosin : Jurnal Ilmiah Kebidanan. 2022 Feb 1;9(1):40–50.
4. Nsugbe E, Obajemu O, Samuel OW, Sanusi I. Application of noninvasive magnetomyography in labour imminency prediction for term and preterm pregnancies and ethnicity specific labour prediction. Machine Learning with Applications. 2021 Sep;5:100066.
5. Jørgensen JS, Weile LKK, Lamont RF. Preterm labor: current tocolytic options for the treatment of preterm labor. Expert Opin Pharmacother. 2014 Apr 24;15(5):585–8.
6. World Health Organization. World health statistics overview 2019: monitoring health for the SDGs, sustainable development goals. World Health Organization 2019. 2019;28.

7. Waks AB, Martinez-King LC, Santiago G, Laurent LC, Jacobs MB. Developing a risk profile for spontaneous preterm birth and short interval to delivery among patients with threatened preterm labor. Am J Obstet Gynecol MFM. 2022 Nov;4(6):100727.
8. Phung J, Wang CA, Reeders J, Chan EC, Riveros C, Zakar T, et al. Preterm labor is a distinct process from term labor following computational analysis of human myometrium. Am J Obstet Gynecol. 2022 Jan;226(1):106.e1-106.e16.
9. Stinson LF, Payne MS. Infection-mediated preterm birth: Bacterial origins and avenues for intervention. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2019 Dec;59(6):781–90.
10. Garcia-Flores V, Romero R, Peyvandipour A, Galaz J, Pusod E, Panaitescu B, et al. A single-cell atlas of murine reproductive tissues during preterm labor. Cell Rep. 2023 Jan;42(1):111846.
11. Michikata K, Sameshima H. Cerclage 1: General. In: Preterm Labor and Delivery. Springer; 2020. p. 161–5.
12. White H, Morton VH, Stock SJ, Lavender T. Preterm labour decision-making and experiences of care for women and clinicians (QUIDS Qualitative): A qualitative exploration. Sexual & Reproductive Healthcare. 2019 Oct;21:95–101.
13. Ibrahim M, Mohamed Ramy AR, Younis MAF. Progesterone supplementation for prevention of preterm labor: A randomized controlled trial. Middle East Fertil Soc J. 2010 Jan;15(1):39–41.
14. Shin SY, Kim MS, Kim YG, Kang D, Choi HY, Pae JY, et al. Characteristics of amniotic mesenchymal stromal cells derived from term and preterm labor. Taiwan J Obstet Gynecol. 2022 Jan;61(1):51–6.
15. Mahendroo M. Cervical remodeling in term and preterm birth: insights from an animal model. Reproduction. 2012 Apr;143(4):429–38.
16. McCarthy R, Martin-Fairey C, Sojka DK, Herzog ED, Jungheim ES, Stout MJ, et al. Mouse models of preterm birth: suggested assessment and reporting guidelines. Biol Reprod. 2018 Nov;99(5):922–37.
17. Grohmann E, Christie PJ, Waksman G, Backert S. Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol. 2018 Feb;107(4):455–71.
18. Mondal IH. Protective Textiles from Natural Resources. Elsevier Science; 2022. (The Textile Institute Book Series).
19. Hamida F, Aliya LS, Syafriana V, Pratiwi D. Escherichia Coli Resisten Antibiotik Asal Air Keran Di Kampus ISTN. Jurnal Kesehatan. 2019 Jun 16;12(1):63–72.
20. Elovitz MA, Mrinalini C. Animal models of preterm birth. Trends Endocrinol Metab. 2004 Dec;15(10):479–87.
21. Gomez-Lopez N, Arenas-Hernandez M, Romero R, Miller D, Garcia-Flores V, Leng Y, et al. Regulatory T Cells Play a Role in a Subset of Idiopathic Preterm Labor/Birth and Adverse Neonatal Outcomes. Cell Rep. 2020 Jul;32(1):107874.
22. Masfufatun M, Tania POA, Raharjo LH, Baktir A. Kadar IL-6 dan IL-10 Serum pada Tahapan Inflamasi di Rattus norvegicus yang terinfeksi Candida albicans. Jurnal Kedokteran Brawijaya. 2018 Feb 28;19–23.