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# The Effect of Exposure to *Escherichia coli* with Low Molecular Weight Hyaluronan, High Molecular Weight Hyaluronan, Hyaluronidase in the Cervix Premature Pregnant Model of Balb/C Mice

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**KEYWORD**
Hyaluronan; Hyaluronidase; Preterm

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## ABSTRACT

**Introduction:** Infection is the biggest cause of preterm labor, about 25-40%, and ascending infection through the vagina is the most common route of infection. This study aims to evaluate the level of Low Molecular Weight Hyaluronan (LMWHA), level of High Molecular Weight Hyaluronan (HMWHA), and the level of the enzyme hyaluronidase in Balb/c premature labor due to infection with Escherichia coli model mice.

**Methods:** This study used an actual experimental design carried out in the laboratory in vivo on balb/c mice exposure to Escherichia coli with a randomized post-test only with a control group design. In the premature model of exposure to Escherichia coli balb/c mice on the cervix of 109 CFU/ml, cervical samples were taken and the levels of LMWHA, HMWHA, and hyaluronidase were measured using spectrophotometry. This study was divided into 2 groups. Control group and treatment group.

**Results:** Low Molecular Weight Hyaluronan (LMWHA) and of the hyaluronidase enzyme levels in premature Balb/C cervical mice exposed to *E. coli* were higher than in Balb/C cervical mice. High Molecular Weight Hyaluronan (HMWHA) levels in premature Balb/C cervical mice exposed to *E. coli* were lower

**Conclusion:** In the cervix of the infected by *E. coli* mice model, LMWHA and hyaluronidase enzymes increased and HMWHA decreased.

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## INTRODUCTION

The rate of preterm delivery ranges from 5-18% of the total infant birth rate worldwide. Premature labor is delivery that takes place from 20 weeks of gestation until before 37 weeks calculated from the first day of the last menstrual period. Infection from the vagina to the cervix, the infection defense mechanism, plays an important role in maintaining proper cervical remodeling. Complications of preterm labor can occur in the mother, as well as in the baby. Premature babies have a greater risk of disease complications than normal babies. Complications are divided into two types, namely short-term complications such as several disorders of body organ function, such as the heart, brain, respiratory tract, and digestive tract, as well as immune disorders and difficulty regulating body temperature, and long-term complications such as jaundice, due to impaired liver function [1,2].



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In principle, the mechanism of preterm labor is the same as normal delivery. However, in preterm labor, various intermediate modulatory stimulations such as infection/inflammation (chorioamnionitis and uteroplacental insufficiency) cause changes in the collagen fibril architecture resulting in rupture of the amniotic membrane and cervical remodeling which is necessary for the labor process [1]. Histologically, the cervix is composed of epithelial and extracellular matrix. The endocervical canal is lined by a layer of columnar epithelium that produces mucus. This layer extends to the vaginal squamous epithelium and forms the squamocolumnar junction, an anatomical location that is prone to malignancy. Histologically, the epithelial cells of the endocervical tract appear tall and slender, firmly attached. The cervix has no glands. In contrast, the folds of columnar epithelium produce cervical mucus and serve as a storage site for spermatozoa after sexual intercourse. Squamous metaplastic processes often close this gap, which clinically manifests as Nabothian follicles [3,4].

Biochemically, the labor mechanism is considered a physiological inflammatory reaction induced by various inflammatory mediators. In principle, the mechanism of preterm labor is the same as normal delivery. However, in preterm labor, stimulation of intermediate modulators may result from infection or inflammation, such as chorioamnionitis and uteroplacental insufficiency. At the term cervix, there is a decrease in fibrin causing fluid retention, resulting in an edematous cervix. His process the pathological softening of cervical tissue is followed by the infiltration of inflammatory cells similar to an inflammatory reaction. This event is thought to occur more rapidly in preterm labor [1].

In preterm labor, as in normal labor, three important elements play a role: cervical ripening, decidua activation, and uterine contractions. The mechanism of cervical remodeling in preterm labor based on the dysfunction that occurs in the cervix can be explained as follows [5,6]. Infection or inflammation appears to be a significant factor in preterm labor. There are several hypothesized mechanisms of inflammation-induced preterm birth, including that the vagina is first exposed to bacteria and then followed by ascending infection through the cervix to the decidua and myometrium. Infection in the decidua results in a maternal inflammatory response and entry of bacteria and/or maternal inflammatory mediators into the fetal membranes, amniotic fluid, and ultimately the fetus. Intrauterine inflammation upregulates contraction-related proteins and inflammatory mediators in the uterus. Similarly, intrauterine inflammation triggers cervical remodeling and maturation [7].

The extracellular matrix has an important role in regulating the development, function, and homeostasis of all eukaryotic cells [8]. The extracellular matrix can undergo dynamic changes in response to environmental stimuli, allowing tissues to maintain homeostasis. The extracellular matrix is produced by cells. The macromolecular structure of the extracellular matrix is different in composition in each tissue [9].

Hyaluronan is a linear polysaccharide with repeating disaccharides D-glucuronic acid and N-acetyl-D-glucosamine. Expressed in the extracellular matrix on the cell surface and inside cells. When broken down into simple polysaccharides, Hyaluronan has various biological functions. Hyaluronan interacts with various proteins or proteoglycans to regulate the extracellular matrix and maintain tissue homeostasis. The unique physical and mechanical properties of hyaluronan contribute to the maintenance of tissue hydration, mediation of the diffusion of solutes through the extracellular space, and the lubrication of certain tissues. The diverse biological functions of hyaluronan are manifested through complex interactions with matrix components and surrounding cells [10].

Hyaluronan plays a role in the differentiation of fibrocytes in injured tissue. Monocytes will infiltrate the tissue and differentiate into fibroblast-like cells called fibrocytes. As an extracellular matrix, hyaluronan can be High Molecular Weight Hyaluronan (HMWHA; 8 x 105 Da - 2 x 106 Da). After an injury, HMWHA will decompose into Low Molecular Weight Hyaluronan (LMWHA; 0.8 – 8 x 105 Da) [11]. Unlike other glycosaminoglycan molecules synthesized in the Golgi apparatus, hyaluronan is synthesized at the plasma membrane by specialized membrane proteins, Hyaluronan Synthases (HASs). There are three HAS isozymes in mammalian species namely HAS1, HAS2, and HAS3 each having two different binding domains. An interesting question is why the three different isozymes for the synthesis of hyaluronan, although these three enzymes have the same structure, differ in the ability to synthesize hyaluronan. HAS1 had a significantly higher Michaelis-Menten constant (Km), compared to HAS2 and HAS3, indicating that HAS1 had a slower rate of synthesizing hyaluronan compared to other syntheses. Therefore, the expression of various HAS isozymes is likely to be a good control system for the effective mediation of diverse cell behaviors. HAS1 and HAS2 are capable of producing large hyaluronan (up to 2000 kDa), Hyaluronan produced by HAS3 has a smaller molecular mass (100–1000 kDa) [12].

The temporal pattern of expression shows increased hyaluronidase activity and increased levels of low molecular weight hyaluronan at the time of delivery and immediately after delivery. Cervical hyaluronidase enzyme activity increases significantly during labor. Increased hyaluronidase activity is associated with a shift from large molecular weight hyaluronan during cervical ripening to an increase in lower molecular weight hyaluronan in the postpartum period [13].



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The cervical hyaluronan-deficient mice, through deletion of the hyaluronan synthase gene, could interfere with cell signal transduction required for epithelial differentiation and mucosal barriers, resulting in increased susceptibility to infection-mediated risk of preterm labor. The mechanism of infection by bacteria that produce hyaluronidase, namely Group B streptococcus, through a mechanism of action by forming hyaluronan bonds with TLR 2 / 4 receptors to prevent the inflammatory response needed to fight ascending infection which results in the risk of preterm labor [13].

Small molecular weight hyaluronan-activated TLR signal transduction pathways differ from pathogenic-derived ligands such as lipopolysaccharides. Regardless of the cause of preterm labor, premature cervical changes precede preterm labor. Understanding the molecular mechanisms of cervical remodeling is an important investigation pathway to determine the mechanism of preterm labor and thus will assist in the development of detection and preterm delivery [14]. The ability of hyaluronan to compete with lipopolysaccharides will lead to infection-mediated preterm labor. An assessment can be made of cervical mucus from pregnant women to determine whether the increased activity of hyaluronidase and small molecular weight of hyaluronan correlates with gestational development and the degree of cervical ripening as determined by the Bishops score [15].

Infection is one of the most important and potentially preventable causes of preterm labor. Infection is detected in 25 to 40% of all cases of preterm labor. Multiple loci of infection are associated with preterm labor, namely systemic infections (influenza, sepsis, pneumonia) and intrauterine infections (intraamniotic and extra amniotic). Although the ability of systemic infections to cause preterm labor is very high, the occurrence of systemic infections during pregnancy is relatively rare, presumably related to faster and more accurate diagnosis and increased use of prophylactic antibiotics [16]. Previous studies have established that uropathogenic E. coli is the main cause of urinary tract infections (UTIs). E coli can attach to vaginal epithelial cells and can cause ascending infection during pregnancy and cause preterm labor [17].

Pregnant mice that were inoculated with Escherichia coli vaginally with two bioluminescent strains of Escherichia coli were found to have a developmental journey up to the cervix, into the uterus, and across the placenta, and then to the fetus. This ascending infection results in preterm labor, decreased live births, and postnatal brain inflammation, suggesting a mechanical link between ascending bacterial infection and poor pregnancy outcomes [18].

This study aims to prove that there is an increase in the level of Low Molecular Weight Hyaluronan (LMWHA), a decrease in the level of High Molecular Weight Hyaluronan (HMWHA), and an increase in the level of the enzyme hyaluronidase in Balb/c mice that experienced premature labor due to infection with Escherichia coli.

## MATERIAL AND METHODS

### Study Design

The study design used in this study was true experimental with a post-test-only control group design approach. The treatment in this study was the administration of Escherichia coli as a preliminary study. Preliminary research aims to see the effect of giving Escherichia coli to the cervix on preterm labor so that it can produce premature model mice which can later be used as a research model for preterm labor using Escherichia coli following the culture provided by the Microbiology laboratory of the Faculty of Medicine, Universitas Brawijaya. The aims were to evaluate the delivery time of Balb/C mice after being given Escherichia coli treatment.

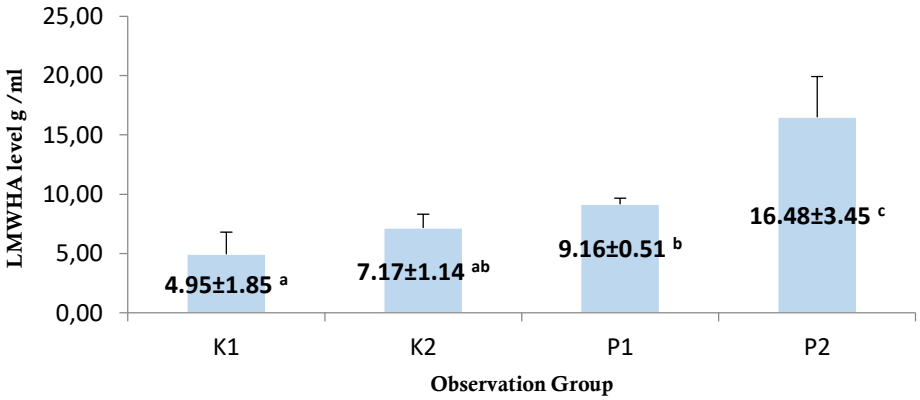



**Fig. 1** Histogram Mean Levels of Low Molecular Weight Hyaluronan in the Group Control and Group Treatment with Administration of Escherichia coli 109 CFU/ml



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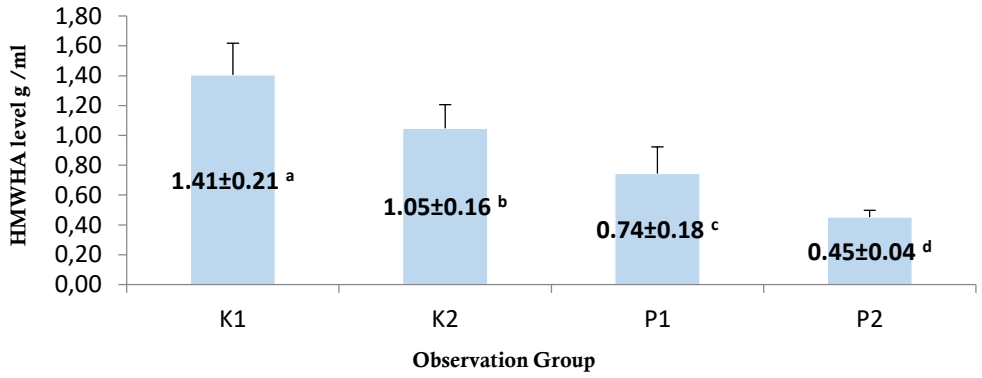



**Fig. 2** Histogram The Average Level of High Molecular Weight Hyaluronan in the Group Control and Group Treatment with Administration of Escherichia coli 109 CFU/ml

The preliminary study consisted of 2 stages, the first stage was to determine the dose of Escherichia coli used in Balb/C mice to produce preterm labor; while the second stage was used as a reference for dosing in Balb/C mice and then evaluated the average delivery rate of Balb/C mice after being treated with Escherichia coli. While the phenomena that occurred due to the treatment from the researchers were only observed after the treatment was given in this study were the levels of LMWHA, HMWHA, and levels of the hyaluronidase enzyme. After the Balb/C mice were performed with Sacrifice, cervical tissue was taken, using spectrophotometry, and the levels of LMWHA, HMWHA, and hyaluronidase were calculated. The resulting data were analyzed using SPSS software for Windows 25.0.

**Sample**

This study used female and male Balb/C mice for fertilization. Then the group was divided into 2 groups of pregnant balb/C mice. Control group and treatment group. The control group was divided into 2 groups, K1: Mice pregnant on the 15th day were sacrificed; K2: Mice pregnant at term followed by delivery, then sacrifice was performed. The treatment group was divided into 2 groups, P1: Pregnant mice on the 15th day were given Escherichia coli on the cervix 109 CFU/ml continued until 38 hours after Escherichia coli was given and then sacrificed; P2: Pregnant mice on the 15th day were given Escherichia coli the cervix was 109 CFU/ml in the cervix followed by parturition, then the sacrifice was performed, followed by labeling and examination using spectrophotometry to determine the levels of LMWHA, HMWHA, and hyaluronidase.

This study used a sample of 28 healthy pregnant female mice. Therefore, the inclusion criteria of the research subjects were mice (Balb/c) of the female sex, aged 60 days, with a body weight of 20-25 grams, and in a healthy condition characterized by active movements. Meanwhile, the exclusion criteria were if the mice looked sick (not moving) before treatment or died during the research process. Balb/c mice were from the *Laboratorium Penelitian dan Hewan Coba* (LPHC) of the Faculty of Medicine Brawiijaya University, Malang, East Java. Escherichia coli was obtained from extraintestinal Escherichia coli isolates carried out in the Microbiology laboratory of the Faculty of Medicine, Universitas Brawijaya Malang.

The cervical preparations for mice and measurements of LMWHA and HMWHA levels as well as hyaluronidase enzymes, were carried out in the Physiology laboratory of the Faculty of Medicine, Universitas Brawijaya Malang.

Data analysis using normality test and homogeneity test, if the data is normal and homogeneous with the Shapiro Wilk test, then the comparison test using the independent sample t-test is continued, but if the data is not homogeneous/abnormal then the Mann-Whitney test is continued. All calculations are done with the help of SPSS software for Windows 25.0.

**Ethics**

All techniques in this study were carried out in compliance with the appropriate manuals and regulations and were approved by the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University/ dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia, with ethic code number: 400/177/K.3/302/2021.

**Statistical Analysis**

Statistical analysis was analyzed using SPSS Version 25.0 for Windows. Furthermore, Data analysis using the normality test and homogeneity test. If the data is



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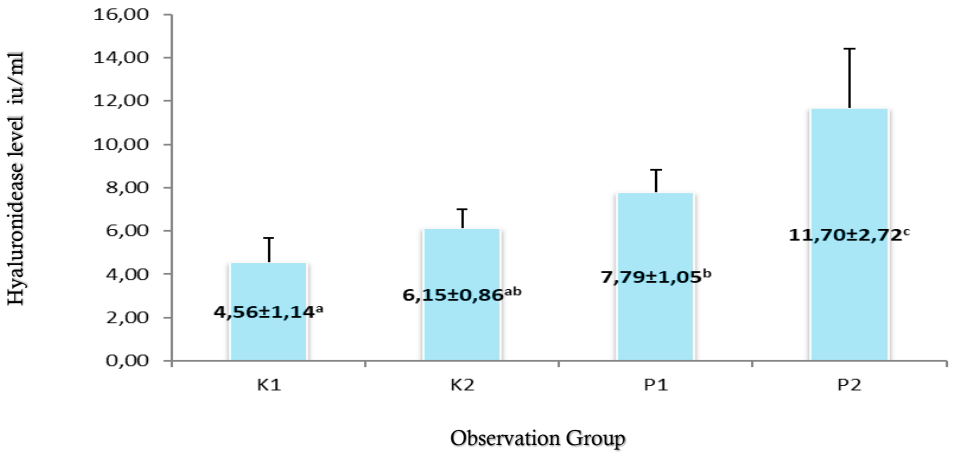



**Fig. 3** Histograms of Hyaluronidase Enzyme Levels in the Group Control and Group Treatment with Administration of Escherichia coli 109 CFU/ml

normal and homogeneous with the Shapiro wilk test, then the comparison test using the independent sample t-test is continued, but if the data is not homogeneous/abnormal, then the Mann-Whitney test is continued.

## RESULTS

The study design used in this study was true experimental with a post-test-only control group design approach. This study is an in vivo study using Balb/C mice as experimental animals. 28 pregnant Balb/C mice were divided into 2 parts, namely the control group and the treatment group. Each group was further divided into 2 small groups with each group consisting of 7 individuals. Groups K1 and K2 are grouped without treatment, while groups P1 and P2 are treatment groups, namely the group that was given Escherichia coli on the cervix.

**Comparison test results from rate Low Molecular Weight Hyaluronan**

Comparison of average LMWHA levels in all four-group sample observation with using the Anova test oneway obtained there is a meaningful difference. This thing showed with p-value=0.000<∝. As for the fourth compared group LMWHA levels are group control 1 (K1: Mice were pregnant on the 15th day later done sacrifice (not yet parturition ), group control 2 (K2: Mice pregnant term followed until with parturition then done sacrifice), group treatment 1 (P1: 15$^{th}$-day pregnant mice were given Escherichia coli at 38 hours, mice done sacrifice), and group treatment 2 (P2: 15$^{th}$-day pregnant mice were given Escherichia coli followed by until with parturition then performed sacrifice).

Then, if the Anova test oneway obtained results there is a meaningful difference, so next with comparison test multiple (Multiple Comparison) with Tukey's test was selected. Next average LMWHA levels in all four group samples the served complete shown in the histogram (Fig.1). Fig. 1 shows the histogram of the mean LMWHA levels in group control 1 (K1 = Mice pregnant 15 days later done sacrifice not yet parturition), group control 2 (K2 = Mice pregnant term followed until with parturition then done sacrifice), group treatment 1 (P1 = 15$^{th}$-day pregnant mice were given Escherichia coli, at 38 hours mice done sacrifice), and group treatment 2 (P2 = 15$^{th}$-day pregnant mice were given Escherichia coli followed until with parturition then sacrifices are made ). Shown in the picture is the stem average The highest LMWHA levels were in the P2 group (16.48±3.45c) and the lowest was in the stem average LMWHA levels in group K1 (4.95±1.85a). This thing means that administration of Escherichia coli in preterm pregnant mice resulted in enhancement of LMWHA levels.

**Comparison test results High Molecular Weight Hyaluronan levels**

Comparison of average HMWHA levels in all four-group sample observation using the Anova test oneway obtained there is a meaningful difference. This thing showed with p-value=0.000<∝. If the Anova test oneway obtained results there is a meaningful difference, so next with the Tukey test. Next average HMWHA levels in all four group samples the served by complete shown in the histogram image (Fig. 2). Fig. 2 shows the histogram of the mean HMWHA levels in group control 1 (K1: Mice were pregnant on the 15th day later done sacrifice (not yet parturition), group control 2 (K2: Mice pregnant term followed until with parturition then done sacrifice), group treatment 1 (P1: 15$^{th}$-day pregnant mice were given E.coli, at 38 hours, mice done sacrifice), and group treatment 2 (P2: 15$^{th}$-day



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pregnant mice were given Escherichia coli followed by until with parturition then sacrifices are made). As shown in the picture the stem average HMWHA levels were highest in the K1 group (1.41±0.21a) and the lowest in the stem average HMWHA levels in group P2 (0.45±0.04d). This thing means that administration of Escherichia coli in preterm pregnant mice resulted in decreased HMWHA levels.

**Comparative Test Results of Hyaluronidase Enzyme Levels**

In the comparison of the mean levels of the hyaluronidase enzyme in the four groups of observational samples using the one-way Anova test, it was found that there was a significant difference. This is indicated by the value of p-value=0.000<∝. If the one-way Anova test results in a significant difference, then it is continued with the Tukey test.

The next average rate of hyaluronidase enzymes in the four sample groups is presented in full as shown in the histogram (Fig. 3). Fig. 3 shows the histogram of the average levels of the hyaluronidase enzyme in group control 1 (K1: Mice pregnant 15 days later done sacrifice (not yet parturition)), group control 2 (K2: Mice pregnant term followed until with parturition then done sacrifice), group treatment 1 (P1: 15$^{th}$-day pregnant mice were given E.coli at 38 hours, mice done sacrifice), and group treatment 2 (P2: 15$^{th}$-day pregnant mice were given Escherichia coli followed by until with parturition then sacrifices are made ). It can be seen in the figure that the average bar of hyaluronidase enzyme levels was highest in the P2 group (11.70±2.72c) and the lowest was in the bar of the average level of the hyaluronidase enzyme in the K1 group (4.56±1.14a). This means that the administration of Escherichia coli in preterm pregnant mice resulted in an increase in the rate of hyaluronidase enzyme.

## DISCUSSION

Infection Intrauterine caused by bacteria mentioned is the reason main labor premature because of infection. The existence infection the established through inspection histopathologically, but clinical criteria, biochemical, and microbiological has used to enforce the diagnosis of infection intrauterine [15,17].

The study before proved that hyaluronan has a role double on the cervix During pregnancy and childbirth. The difference in the role of hyaluronan in the epithelium mucosal and non- mucosal cervix, as well as matrix extracellular, is based on heavy molecules and types of bonds Specific to hyaluronan. Hyaluronan is secreted and Becomes mucus cervix. During the phase of maturation, the cervix is considered to become lubrication required for a past fetus. From research, they obtained that not only lack of hyaluronan levels in the cervix but also increase LMWHA improves the risk of birth premature consequence infection ascendance. LMWHA has two functions that are reason loss integrity network and compliance increasingly network increase along with time approach childbirth and existence uterine contractions, and modulate inflammation [5].

Mice that didn't modify the Hyaluronan Synthase-2 (HAS2) gene and administered treatment administration of Escherichia coli experienced labor prematurely by 50%. In two groups of mice than others, with level different HMWHA deficiencies, the numbers of premature labor are 100% and 92%. Lack of HMWHA improves the risk of the premature birth consequence of ascendingly infection. The role of hyaluronan differs in the size of different molecules. Findings in mice this possibility will lead to research on women pregnant to determine if a drop in hyaluronan level production in the ducts reproduction part lower is a factor risk of premature labor. The cleavage of HMWHA into LMWHA is regulated by the hyaluronidase enzyme encoded by five genes, Hyal 1–5 [5].

In our research, we found LMWHA levels were highest in the group treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and followed by until with parturition, followed group treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and sacrificed after 38 hours of administration then followed group control mice Balb /C pregnancy that followed until parturition. The smallest LMWHA result in the group mice Balb /C is pregnant on the 15th day by sacrifice. This thing explained that in the condition parturition occurs enhancement LMWHA levels and treatment administration of Escherichia Coli 109 CFU/ml to the cervix result in enhancement LMWHA levels are increasing high.

This thing in accordance with research concluded that the cervix in pregnancy term similar to edematous tissue that has inflammation. The maturation cervix in rabbits accelerated with the administration to the cervical of LMWHA (400 kDa). Because of it, LMWHA, which is in the cervix since the 15th day of pregnancy and more the amount at the time of childbirth, by no direct cause of maturation uterine cervix. The phase labor occurs at the highest rate of LMWHA, whereas in the post-partum phase it occurs repair that is LMWHA degradation and phagocytosis, fragment versican and collagen cell immunity by neutrophils (Neu) and macrophages[18,19].



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**Decrease in High Molecular Weight Hyaluronan Levels in the Cervix Mice Experiencing Balb/C Labor Premature because Infected with Escherichia coli**

In our study, we found the lowest HMWHA levels in the group treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and followed by until with parturition, followed bygroup treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and sacrificed after 38 hours of administration then followed group control mice Balb /C pregnancy that followed until parturition. HMWHA results were high in the group mice Balb /C is pregnant on the 15th day by sacrifice. This thing explains that in the condition, parturition occurs to drop in HMWHA levels and treatment administration of Escherichia Coli 109 CFU/ml to the cervix results in decreased HMWHA levels, getting lower than before. Hyaluronan on the cervix mice increases from 7.4 nmol/mg to 24.6 nmol/mg at the age 18$^{th}$ days of pregnancy. Similar to research conducted before that HMWHA increasingly increases at the end of pregnancy, but at HMWHA delivery will be degraded to become LMWHA [5,19].

Hyaluronan plays a role in the differentiation of cell Fibocytes on injured tissue. Monocytes will infiltrate the network and differentiate Becomes Cells that resemble fibroblasts are called cell fibrocytes. As matrix extracellularly, hyaluronan can be in the form of High Molecular Weight Hyaluronan (HMWHA; 8 x 105 Da - 2 x 106 Da). After suffering an injury, such as the occurrence of the infection process, HMWHA will be unraveled to Low Molecular Weight Hyaluronan (LMWHA; 0.8 – 8 x 105 Da) [11].

The study before, evaluated the function of hyaluronan in cervix and mucus cervicovaginal During pregnancy and occurrence of labor premature related infection using the mouse model GMO with depletion of the Hyaluronan (Has-2) -forming gene. The result of the study found in normal delivery increases in cervical HMWHA at the end of pregnancy cause distensibility of cervix maximal and disorganized very good collagen important for successful delivery. While in childbirth premature related infection using the mouse model GMO with Hyaluronan Synthase-2 (Has-2) gene depletion evaluates the role function epithelium cervix in childbirth and events labor mediated premature infection. It was found that the cervical epithelium appeared irregular and had defects in occluding expression, as well as interfered with the formation of tight junction proteins. So that could be concluded that HMWHA is needed to maintain the structure and integrity of the epithelium so that could function as a barrier against bacterial infections that cause labor premature. This finding is in accordance with clinical studies i.e There are differences in the nature of cervicovaginal mucus in women at high risk of developing labor prematurely, compared to women who are not at risk [20].

**Increased Hyaluronidase Enzyme Levels in the Cervix Mice Experiencing Balb/C Labor Premature because Infected with Escherichia coli**

In our research, we found the highest hyaluronidase levels in the group treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and followed by until with parturition, followed group treatment mice Balb /C pregnant on the 15th day given Escherichia Coli 109 CFU/ml on the cervix and sacrificed after 38 hours of administration then followed group control mice Balb /C pregnant followed until parturition. The smallest hyaluronidase yield in the group of mice Balb /C is pregnant on the 15th day by sacrifice. This thing explains that in the condition parturition occurs enhancement hyaluronidase levels and treatment administration of Escherichia Coli 109 CFU/ml to the cervix results in enhancement of increased hyaluronidase high levels.

Hyaluronan plays a role important in the process of pregnancy and childbirth. Approaching increased hyaluronan delivery in the cervix by about 1.0% of heavy dry cervical. Successful vaginal delivery is related to the role of hyaluronan in the ripening process cervical. Humans and mice produce hyaluronidase enzyme to parse the HMWHA that is inside the cervical, exogenous hyaluronidase is one method used to induce maturation cervix and childbirth. Interestingly, vaginal inoculation with Escherichia coli that produces hyaluronidase causes enhancement levels of birth prematurely in mice. Intracervical hyaluronidase injection shortens the duration of childbirth. How it works hyaluronidase enzyme does not have an effect directly on activity myometrium but character local to the cervix so that profitable by the potential that shortens duration labor without enhancement risk laser cervix and/ or ruptures of the uterine wall [21,22].

This study about the role of hyaluronan has been many developed in the medical world. Various roles of hyaluronan have been explored, for example in the eye, hyaluronan fluid is used when operating cataracts and glaucomas as fluid physiological, then in orthopedics, hyaluronan can be used in therapy Osteoarthritis as synovial fluid, in the plane dermatology could be used as the useful substance for skin regenerate, lately this use of Hyaluronan a lot researched in therapy neoplastic for preventing active division cell neoplastic. In the field of obstetrics and gynecology study regarding Hyaluronan is still very limited. Whereas as a component matrix extracellularly, hyaluronan is present in almost all component bodies. The hyaluronan content is increasing reduce along with increasing age. In a baby the amount of hyaluronan reaches 80% of the whole



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component body, while at age carry on over 60 years, hyaluronan levels only reached 20% [23].

Limited studies about hyaluronan in the field of obstetrics and gynecology still have many things that can be researched. With knowing the role of hyaluronan in cervical remodeling in labor premature, hope in future day could be researched more carry on about the management and prevention labor related premature with rate hyaluronan on the cervix. So that in the end, you can conclude about the application of therapeutic prevention labor premature through mechanism settings transduction signal matrix extracellular especially hyaluronan levels in cervical remodeling [3].

The final results of our study showed that the treatment in mice Balb /C with giving Escherichia coli to the cervix pregnant mice caused mice parturition premature. Treatment administration of Escherichia coli can increase LMWHA levels in preterm pregnant mice compared to prematurely pregnant mice without Escherichia coli and can increase LMWHA levels in pregnant mice that experienced premature labor compared to mice that were pregnant without Escherichia coli. LMWHA levels in preterm pregnant mice that were given Escherichia coli were higher than in pregnant mice that underwent term parturition. The highest levels of LMWHA were found in pregnant mice that experienced premature labor.

Treatment administration of Escherichia coli can lower HMWHA levels in preterm pregnant mice compared to prematurely pregnant mice without Escherichia coli administration and can lower HMWHA levels in pregnant mice that experienced premature labor compared to mice that were pregnant without Escherichia coli. The levels of HMWHA in preterm pregnant mice that were given Escherichia coli were lower than those of pregnant mice that underwent term parturition. The lowest levels of HMWHA were found in pregnant mice with premature labor.

Administration of Escherichia coli can increase levels of hyaluronidase enzyme in preterm pregnant mice compared to preterm pregnant mice without Escherichia coli administration and can increase levels of hyaluronidase enzyme in pregnant mice that undergo premature labor compared to pregnant mice that experience term parturition. The levels of the hyaluronidase enzyme in preterm pregnant mice that were given Escherichia coli were higher than those of pregnant mice that had term parturition. The highest levels of the hyaluronidase enzyme were found in pregnant mice that had parturition premature.

## CONCLUSION

The results of this study were the Levels of Low Molecular Weight Hyaluronan (LMWHA) increased, levels of High Molecular Weight Hyaluronan (HMWHA) decreased, and levels of hyaluronidase enzymes increase in the cervix premature model BALB/C mice exposed to *E. coli* bacteria.

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## CONFLICT OF INTEREST

The authors declared that there was no conflict of interest regarding the publication of this article.

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