

Differences in Grade II Perineal Tears Wound Healing using Fresh Amniotic Membrane in Post Vaginal Delivery Women in Padang Panjang Indonesia

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Abstract

Introduction: Perineal tear is the most common complication of vaginal delivery which has the risk of infection and discomfort, also causes insecurity. The amniotic membrane has been shown to enhance wound healing through acceleration of epithelization, angiogenetic and antibacterial effects. This study aims to determine the difference in perineal wounds healing with or without the use of amniotic membranes.

Methods: This was a cohort study conducted from December 2022 to January 2023 at Padang Panjang Hospital. Patients included were aged 17- 40 years with normal BMI without any comorbid such as diabetes mellitus, hypertension and blood disorders. The procedure was done by the same person at the same place and with the same equipment.

Results: There were 28 patients who were divided into two groups i.e 14 patients who were given fresh amniotic membranes and 14 patients who were not given fresh amniotic membranes. The mean age, parity, last education, body mass index and type of perineal wound were similar between groups. In this study, there was a significant difference between recovery and pain degrees on the 10th day after the procedure between the two groups, while there was no significant difference in the incidence of infection on the 10th day and pain on the 2nd day.

Conclusion: Fresh amniotic membrane improves healing and reduces the pain in perineal wound patients.

Key words: Fresh amniotic membrane, recovery, pain, infection, perineal wound

Perbedaan Penyembuhan Luka Perineum Grade II dengan Penggunaan Selaput Amnion Segar pada Wanita Pasca-Persalinan Pervaginam Di RSUD Padang Panjang Indonesia

Abstrak

Pendahuluan: Robekan perineum merupakan penyebab kedua terbanyak perdarahan postpartum. Selaput amnion telah terbukti meningkatkan hasil penyembuhan luka melalui efek percepatan epitelisasi, angiogenetik dan antibakterial. Penelitian ini bertujuan untuk mengetahui perbedaan penyembuhan pada luka perineum grade II dengan atau tanpa penggunaan selaput amnion.

Metode: Penelitian ini merupakan jenis studi kohort yang dilakukan dari Desember 2022 sampai Januari 2023 di RSUD Padang Panjang. Pasien yang diikutkan adalah pasien berusia 17 – 40 tahun dengan IMT normal tanpa adanya komorbiditas diabetes melitus, hipertensi dan kelainan darah. Penjahitan luka dan pemberian amnion dilakukan dengan operator, lokasi serta alat yang sama menggunakan PGA 2.0 teknik jelujur dan kulit dengan teknik subkutikuler.

Hasil: Terdapat sebanyak 28 pasien yang dibagi menjadi dua kelompok, yaitu 14 pasien yang diberikan selaput amnion segar dan 14 pasien tidak diberikan selaput amnion segar. Rerata usia, paritas, pendidikan terakhir, indeks masa tubuh, dan jenis luka perineum grade II serupa antar kelompok. Pada penelitian ini didapatkan perbedaan yang signifikan antara kesembuhan hari ke-10 dan derajat nyeri hari ke-10 pasca tindakan antara kedua kelompok, sedangkan ada kejadian infeksi hari ke-10 an nyerihari ke-2 tidak ditemukan perbedaan signifikan.

Kesimpulan: Selaput amnion segar meningkatkan kesembuhan dan menurunkan nyeri pada pasien dengan luka perineum grade II.

Kata kunci: Selaput amnion segar, kesembuhan, nyeri, infeksi, luka perineum grade II

Introduction

More than 85% of women undergoing vaginal birth will experience some degree of perineal tearing, with 0.6-11% of all vaginal deliveries resulting in third- or fourth-degree tearing. Fortunately, the incidence of perineal tears decreases with subsequent births, from 90.4% in nulliparous women to 68.8% in multiparous women undergoing vaginal birth.¹ There are 2.7 million cases of perineal tears in parturients and are estimated to increase by 6.3 million by 2050 if midwifery care does not improve. The incidence of perineal rupture in Asia is still high, accounting for 50% of perineal rupture incidence in the world. In Indonesia alone, mothers who experience perineal rupture at the age of 25-30 years account for a proportion of 24%, while at the age of 32-39 years it is 62%. Perineal tears are the most common complication in childbirth.^{2,3} Although perineal tears are a minor complication, women with perineal tears can experience physical, psychological and social problems.^{4,5}

Acute wound healing, triggered by tissue injury, consists of hemostasis, inflammation, proliferation, and remodeling phases.⁶ Amnions improve the results of wound healing. The healing effect of the amnion is due to chemical and biomechanical properties such as antibacterial and angiogenetic effects, accelerating and protecting epithelialization as well as stimulating granulation and tissue formation, stimulating neovascularization, reducing pain, healing without scarring, no immunological reactions and increasing mobility.^{7,8} However, no research was found regarding the effects of using amniotic membranes on perineal wounds.

The amniotic membrane consists of three layers, the epithelial layer towards the fetus, the basement membrane, and the stroma. The stroma consists of a dense layer, a fibroblast layer, and a rubbery outer layer. Amniotic membrane acts as a highly

biocompatible natural material and is a source of several types of stem cells and powerful growth factors. The application of amniotic membranes has contributed to a better understanding of stem cell biology.⁹ Because of this stem cell content, amniotic membrane is widely used as an additional procedure in surgical procedures to accelerate tissue healing.

Based on the description above, the author is interested in learning more about the differences in healing of grade II perineal wounds with the use of fresh amniotic membrane in women after vaginal delivery at Padang Panjang Regional Hospital. This is because Padang Panjang Regional Hospital is one of the FK Unand teaching hospitals with the highest number of births. Padang Panjang Regional Hospital had a figure of 137 cases.¹⁰ Padang Panjang City itself has geographical advantages, namely a relatively small area and easy to access.

Methods

This type of research is an analytical study with a cohort study research design with treatment to assess whether there is a difference in healing, level of infection, level of pain in grade II perineal wounds given fresh amniotic membranes and those not given fresh amniotic membranes. The research was conducted at Padang Panjang Regional Hospital in December 2022 - January 2023. The population in this study was all patients with vaginal births at Padang Panjang Regional Hospital in December 2022 - January 2023. Based on calculations, the minimum sample size for each group was 12 people. Correction or increasing the number of samples is based on the prediction of 20% drop out samples from the research, so that the sample size for each research group is 14 people and the total research sample is 28 people.

The independent variable of this study

was the use of fresh amniotic membrane. Meanwhile, the dependent variables used are wound healing, wound infection, wound pain. Grade II perineal healing is divided into 2 categories: 1) Dry wounds: wounds that no longer ooze fluid and 2) Wet wounds: wounds that still ooze fluid other than pus (serum or blood). Grade II perineal wound infections are divided into 2 categories: 1) Yes: There are 5 signs of infection or pus in the wound and 2) No: there are no 5 signs of infection or pus in the wound. Grade II perineal wound pain is categorized using VAS (Visual analog scale): 1) 0 (no pain), 2) 1-3 (mild), 3) 4-6 (moderate), and 4) 7-10 (severe).

Results

Characteristics of vaginal delivery patients with grade II perineal wounds

In Table 1 above, the treatment group (given fresh amniotic membrane) had a mean age of 27.1 ± 4.0 years with the highest parity being nulliparous (64.3%), highest education high school (57.1), overall BMI normoweight with the type of episiotomy wound was

64.3% and grade 2 perineal tears were 35.7%. Meanwhile, the control group (without fresh amniotic membrane) had an average age of 27.5 ± 5.1 years with the highest parity being nulliparous (64.3%), 50% high school graduate, BMI 100% Normoweight, the same type of episiotomy wound and grade 2 perineal tear with the treatment group.

Healing of grade II perineal wounds on the 10th day in patients who given fresh amniotic membranes and who not given fresh amniotic membranes

Thirteen patients (92.9%) had grade II perineal wounds which were already dry in the group given fresh amniotic membrane, one patient (7.1%) found the wounds were still wet. In the control group, there were the same number of patients with dry and wet wounds, seven patients (50%).

The incidence of grade II perineal wound infection on the 10th day in patients who given fresh amniotic membranes and who not given fresh amniotic membranes

Table 1 Characteristics of vaginal delivery patients with grade II perineal wounds at Padang Panjang Regional Hospital

Characteristics	Treatment Group		Control Group	
	Mean ± SD	f(%)	Mean ± SD	f(%)
Age (years)	27,1 ± 4,0		27,5± 5,1	
Parity				
Nulliparous		9 (64,3)		9 (64,3)
Multiparous		5(35,7)		5(35,7)
Education				
Elementary School		1(7,1)		1(7,1)
Junior High School		0(0)		1(7,1)
Senior High School		8(57,1)		7(50)
College		5(35,7)		5(35,7)
BMI				
Normoweight		14(100)		14(100)
Type of tear				
Episiotomy		9(64,3)		9(64,3)
Grade 2 perineal tear		5(35,7)		5(35,7)

Table 2 The Level of Perineal Wound Pain was Grade II in Patients who were Given Fresh Amniotic Membranes and who were not Given Fresh Amniotic Membranes.

Pain Level	Treatment Group f(%)		Control Group f(%)	
	Day-2	Day-10	Day-2	Day-10
No pain	0(0)	7(50)	0(0)	1(7,1)
Mild	9(64,3)	7(50)	10(71,4)	8(57,1)
Moderate	5(35,7)	0(0)	4(28,6)	5(35,7)
	0(0)	0(0)	0(0)	0(0)

Table 3 Differences in grade II Perineal Wound Healing on Day 10 in Patients who were Given Fresh Amniotic Membranes and those who were not Given Fresh Amniotic Membranes.

	Healingf(%)		*P-Value
	Dry	Wet	
Treatment group	13(92,9)	1(7,1)	0,036
Control group	7(50)	7(50)	

*Chi-Square test

None of the 14 (100%) patients who were given fresh amniotic membrane experienced postpartum infections, while in the control group, one patient (7.1%) experienced postpartum infection, and the rest did not experience infection.

Pain level of grade II perineal wound in patients who were given fresh amniotic membranes and who were not given fresh amniotic membranes

Based on Table 2, the pain level in the treatment group given fresh amniotic membrane shows that nine patients (64.3%) had a mild pain level on the 2nd day postpartum, and five patients (35.7%) had a moderate pain level. Meanwhile, in the control group who were not given fresh amniotic membrane, there were ten patients (71.4%) with mild pain and other four patients with moderate pain.

Based on Table 2, the pain level in the treatment group given fresh amniotic membrane showed that seven patients (50%) felt no

pain after the 10th day postpartum, and seven patients (50%) had mild pain. Meanwhile, in the control group who were not given fresh amniotic membrane, it showed that only one patient (7.1%) no longer felt pain in grade II perineal wounds while the remaining eight patients (57.1%) felt mild pain and other five patients felt moderate pain.

Differences in perineal healing in patients who were given fresh amniotic membranes and who were not given fresh amniotic membranes

Based on Table 3, thirteen patients (92.9%) had grade II perineal wounds that were dry in the group given an amniotic membrane, and one patient (7.1%) had wounds that were still wet, while in the control group, there were several patients with dry wounds and wet had the same number, namely seven patients (50%). The table also shows that there is a statistically significant difference between the healing time for grade II perineal wounds that

Table 4 Differences in the Incidence of Grade II Perineal Wound Infections on Day 10 in Patients who were Given Fresh Amniotic Membranes and those who were not Given Fresh Amniotic Membranes.

	Infeksi f(%)		<i>*P-Value</i>
	Yes	No	
Treatment group	0(0)	14(100)	1,000
Control group	1(7,1)	13(92,9)	

**Chi-Square test*

Table 5 Differences in the Level of Grade II Perineal Wound Pain in Patients who were Given Fresh Amniotic Membranes and who were not Given Fresh Amniotic Membranes on the 2nd Day After the Procedure.

Group	Pain level f(%)				<i>*P-Value</i>
	No pain	Mild	Moderate	Severe	
Treatment	0(0)	9(64,3)	5(35,7)	0(0)	0,686
Control	0(0)	10(71,4)	4(28,6)	0(0)	

**Chi-Square test*

Table 6 Differences in the Level of Grade II Perineal Wound Pain in Patients who were Given Fresh Amniotic Membranes and who were not Given Fresh Amniotic Membranes on the 10th Day after the Procedure

Group	Pain level f(%)				<i>*P-Value</i>
	No pain	Mild	Moderate	Severe	
Treatment	7(50)	7(50)	0(0)	0(0)	0,008
Control	1(7,1)	8(57,1)	5(35,7)	0(0)	

**Chi-Square test*

were given amniotic membranes and those that were not given amniotic membranes (p-value of 0.036).

Difference in the incidence of grade II perineal wound infections on day 10 in patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes.

Based on Table 4, none of the 14 (100%) patients who were given fresh amniotic membrane experienced postpartum infections, whereas in the control group, thirteen patients (92.9%) did not experience infections, and there was one patient (7.1%) experienced a postpartum infection. Based on

the Chi-square test, there was no significant difference in the incidence of infection in grade II perineal wounds in patients who were given amniotic membranes and those who were not given fresh amniotic membranes, namely p-value 1,000.

Differences in the level of perineal wound pain in patients who were given fresh amniotic membranes and who were not given fresh amniotic membranes on the 2nd day after the procedure

Based on Table 5, the pain level in the treatment group given fresh amniotic membrane showed that nine patients (64.3%) had mild pain on the 2nd day postpartum,

and five patients (35.7%) had moderate pain levels. Meanwhile, in the control group who did not receive fresh amniotic membranes, ten patients (71.4%) had mild pain, and other four patients had moderate pain. There is no significant difference regarding the grade II perineal wound pain level in patients who were given amniotic membranes and those without fresh amniotic membranes on day 2 (p-value <0.686).

Differences in the grade II perineal wound pain level in patients who were given fresh amniotic membranes and who were not given fresh amniotic membranes on the 10th day after the procedure

Based on Table 6, the pain level in the treatment group given fresh amniotic membrane showed that seven patients (50%) felt no pain after the 10th day postpartum, and seven patients (50%) had mild pain. Meanwhile, one patient (7.1%) in the control group who did not receive fresh amniotic membrane felt no longer pain in grade II perineal wounds, while eight patients (57.1%) had mild pain, and five other patients had moderate pain. There is a significant difference in the grade II perineal wound pain level in patients who were given amniotic membranes and those without fresh amniotic membranes (p-value <0.008).

Discussion

Differences in healing of grade II perineal wounds on day 10 in patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes.

In this study, 13 patients (92.9%) in the fresh amniotic membrane group had wounds that were dry on the 10th day with only one patient (7.1%) having wounds that were still wet, whereas in the control group there were patients with dry and wet wounds had the

same number, namely seven patients (50%). Previously, Wahyuningtyas had conducted animal model research regarding the use of fresh human amniotic membranes in healing rectovaginal fistulas in eight rabbits and eight dogs and obtained higher healing scores in the group given fresh amniotic membranes. The healing score in the study assessed epithelialization, collagenization, inflammation, neovascularization, necrosis, and granulation tissue.¹¹

The amniotic membrane has anti-inflammatory effects driven by human amniotic epithelial cells (hAEC) and human amniotic mesenchymal stromal cells (hAMSC), which express various antiangiogenic and anti-inflammatory proteins such as IL-1 receptor antagonist (IL-1RA), tissue inhibitors of metalloproteinases (TIMPs)-1, -2, -3, -4, and IL-10.¹² Amniotic membrane stromal matrix has demonstrated the ability to inhibit the expression of potent pro-inflammatory cytokines such as IL-1. The inflammatory cytokine IL-1 mediates the extravasation of phagocytes and lymphocytes to sites of inflammation and plays an important role in the inflammatory cascade. The amniotic membrane contains IL-1RA, which can inhibit immune cell migration. The amniotic membrane also contains IL-10, which can inhibit important inflammatory factors such as TNF- α , IL-6, and IL-8, which promote the migration of neutrophils and granulocytes to the site of inflammation. The amniotic membrane can downregulate pro-inflammatory cellular components. Due to the anti-inflammatory effect and the presence of growth factors in the amniotic membrane, almost all the patients with fresh amniotic membrane had wounds that had dried out due to the shortened inflammation time, whereas in the control group there were still some who had wet wounds.^{10,11,13,14}

Difference in the incidence of grade II perineal wound infections on day 10 in

patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes

The decrease in the incidence of infection in the fresh amnion membrane group occurred because human amnion membranes have antimicrobial properties. The amniotic membrane can provide mechanical protection against organisms (because it is attached directly to the wound). The amniotic membrane has high thrombin activity, which allows for tight and efficient attachment of the amniotic membrane to the wound surface. This tight adhesion allows restoration of lymphatic integrity protecting against exposure to surface debris and bacteria and allowing their removal.¹⁵

The amnion membrane also contains transferrin, bactericidin, β -lysin, lysozyme, and immunoglobulin. These molecules have anti-bacterial effects against group B and A *Streptococcus*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus saprophyticus*, *Lactobacillus*, *Pseudomonas aeruginosa*, and *Acinetobacter*.^{13,14}

In this study, there was no significant difference in the incidence of infection because there was only one incidence of infection in the control group. Infection is affected by many things, one of which is patient hygiene. If hygiene in both groups of patients is equally good, then the incidence of infection can be prevented. However, no infections occurred in the treatment group, which proves that protection is more effective with amnions than without amnions.

Difference in the level of grade II perineal wound pain in patients who were given fresh amniotic membranes and who were not given fresh amniotic membranes on day 2 and day 10 after the procedure

This study found that while there was no significant difference in pain on the

second day following the surgery, there was a significant difference in discomfort on the tenth day. These findings are in line with studies conducted in 2022 by Mathur et al.¹⁶ patients with post-operative oral mucosa abnormalities participated in the trial and received both fresh and freeze-dried amniotic membranes. Both forms of amniotic membrane in this study exhibited effective wound healing on days 7 and 21 following surgery, with a considerable decrease in pain level commencing after 3rd day and reaching VAS 0 on day 7. In this study, there was also no reduction in pain before the 3rd day where the VAS pain scale was still the same as the first day (flat curve).

The fresh amniotic membrane's anti-inflammatory characteristics contributed to the fresh amniotic membrane group's quicker pain scale reduction. As is well known, damage to peripheral nerve terminals causes pain after tissue injury. Accelerated re-epithelialization will result in less free exposure to peripheral nerve terminals and hence, less discomfort.¹³

The inflammatory process was still ongoing on the second day, so the inflammatory cytokines that promote the production of pain-inducing substances like histamine were still localized in the wound area. As a result, there was still no improvement in the discomfort. Even when amniotic membranes are used, there are currently no studies that have demonstrated a remission of pain in wounds on the second day following the surgery. This reveals that the effect of the amnion was not yet considerably obvious on day 2 as was also seen in Mathur et al's study with a pain curve that was still present from days 1 to 3 post-treatment.¹⁷

Research Limitations

This study did not test the effect of fresh amniotic membrane on grade II perineal wounds at the molecular level, so it cannot

definitively prove the theory of amniotic membranes on grade II perineal wounds. In this study, full control of patients was not carried out for 10 consecutive days, so it is not known about the patient's vulva hygiene during follow-up. Apart from that, there are still a few reference studies regarding the application of fresh amniotic membrane to grade II perineal wounds for this study.

Conclusion

1. In this study, the average age was 27 years, most parity was nulliparous, most were high school graduates, overall BMI was normoweight, and the most common type of injury was episiotomy. Actions are performed by the same operator, in the same location, and using the same tools.
2. In this study, all patients with fresh amniotic membranes had wounds that were dry on the 10th day, whereas in the control group there were still wet wounds on the 10th day.
3. In this study, most of the treatment and control group patients had mild pain on the second day after the procedure. On the 10th day, most of the patients in the treatment group had experienced pain resolution, while in the control group, most had not experienced pain resolution.
4. In this study, all patients with fresh amniotic membranes did not experience infection on the 10th day of the wound, while in the control group there were patients who got infection.
5. There was a significant difference between wound healing on the 10th day in patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes.
6. There was no significant difference between post-operative pain on day 2 in patients who were given fresh amniotic membranes and those who were not given

fresh amniotic membranes.

7. There is a significant difference between post-operative pain on day 10 in patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes.
8. There was no significant difference between the incidence of infection on day 10 in patients who were given fresh amniotic membranes and those who were not given fresh amniotic membranes.

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