Reduction of Maternal Calponin 1: Evidence to Support Natural Progesterone Superiority to Nifedipine in the Treatment of Uncomplicated Premature Contraction

Tita Husnitawati Madjid, Tina Dewi judistiani, Iwan Heryawan, Jusuf Effendi
Department of Obstetrics and Gynecology, Faculty of Medicine, Padjadjaran University
Bandung, West Java, Indonesia
Corresponding: Tita Husnitawati Madjid, *Email*: thusnitawati@gmail.com

Abstract
Introduction: Premature contractions precede preterm birth, which is still a challenging subject as it may contribute to neonatal mortality. Treatment is often aimed at providing some time window for lung maturation. This study aims to compare the efficacy of nifedipine versus progesterone in postponing preterm birth and to provide evidence of the biological process by changes in maternal serum calponin 1 level.
Method: An oral dose of 20 mg nifedipine given three times daily was compared to a single dose of 400 mg intra-vaginal natural progesterone in a single-blinded, non-randomized controlled trial. Selected subjects were normal, singleton pregnancies between 28 - 34 weeks of gestational age with premature contraction and intact amniotic membrane. The primary outcome was a reduction in the frequency and strength of contraction, and the secondary measure was the adjustment of serum calponin before and after the intervention.
Results: This finding was supported by reducing calponin levels in maternal sera. Despite the reduction of frequency and strength of contractions occurring in the nifedipine arm, serum calponin level increased, indicating that the myometrial contractility pathway was not completely deterred.
Conclusion: This study revealed that natural progesterone was superior to nifedipine in treating premature contraction. It was also supported by evidence of maternal sera calponin level reduction, indicating disruption of the contraction pathway.

Keywords: Calponin 1, Nifedipine, Premature Contractions, Preterm Birth, Progesterone

Penurunan Serum Calponin 1 Ibu: Bukti yang Mendukung Keunggulan Progesteron Alami dibandingkan Nifedipine dalam Pengobatan Kontraksi Dini Tanpa Komplikasi

Abstrak
Pendahuluan: Kelahiran prematur selalu didahului oleh kontraksi prematur. Hingga saai ini kelahiran prematur masih menjadi topik yang menantang karena dapat berkontribusi pada kematian neonatal. Pengobatan sering bertujuan memberikan waktu untuk pematangan paru-paru. Tujuan penelitian ini adalah untuk membandingkan efektivitas nifedipin versus progesteron dalam menunda kelahiran prematur dan untuk memberikan bukti proses biologis melalui perubahan kadar serum calponin 1 ibu.
Metode: Dosis oral 20mg nifedipin yang diberikan tiga kali sehari dibandingkan dengan dosis tunggal 400mg progesteron alami intra-vagina dalam uji coba *single-blinded non-randomized controlled*. Subjek yang dipilih adalah kehamilan normal, kehamilan tunggal usia kehamilan antara 28 - 34 minggu dengan kontraksi prematur dan selaput ketuban yang utuh. Hasil utama adalah pengurangan frekuensi dan kekuatan kontraksi dan ukuran sekunder adalah penyesuaian serum calponin sebelum dan sesudah intervensi.
Hasil: Temuan ini didukung oleh penurunan kadar calponin dalam serum ibu. Meskipun terjadi pengurangan frekuensi dan kekuatan kontraksi pada lengan nifedipin, kadar serum calponin meningkat yang mungkin mengindikasikan bahwa jalur kontraktilitas miometrium tidak sepenuhnya terhalang.
Kesimpulan: Hasilnya, penelitian ini mengungkapkan bahwa progesteron alami lebih unggul daripada nifedipin dalam pengobatan kontraksi dini. Hal ini juga didukung oleh bukti penurunan kadar serum calponin ibu yang mengindikasikan gangguan pada alur kontraksi.

Kata kunci: Calponin 1, Nifedipine, Kontraksi Dini, Kelahiran Prematur, Progesteron

Introduction

Preterm birth, defined as delivery prior to 37 weeks gestation, is a worldwide epidemic. Preterm birth was estimated to occur in 1 out of every 10 pregnancies worldwide.[1] The psychological, emotional, and financial costs are incalculable as other causes of neonatal deaths. Preterm birth can be further classified as spontaneous preterm birth or medically indicated (iatrogenic) preterm birth.[1] Pathways to spontaneous preterm birth include preterm labor, preterm premature rupture of membranes, and second-trimester spontaneous pregnancy loss.[1] The most potential pathway to reducing preterm birth is to develop effective tools for identifying high-risk groups for preterm labor and premature membrane rupture. Moreover, screening for risk factors was made available through quality antenatal care, including the utilization of cervical length measurement by ultrasound examination and cervical mucous biomarkers such as fetal fibronectin and calponin as an alternative.[2]

Spontaneous preterm births also occur in nulliparous women without identifiable risk factors, and therefore, preventative strategies are limited.[1] There are numerous preterm labor diagnostic criteria and management protocols, but the goal is the same. It is to prevent the delivery of preterm infants before the 37th week of pregnancy, or in less favorable conditions, to provide a window of opportunity to accelerate lung maturity even though preterm infants' deliveries were inevitable.[3] Premature contraction precedes preterm labor, although some cases show that cervical dilation does not always follow premature contraction. Preventing preterm birth requires therapy to eliminate premature contractions.

Progesterone, a naturally acquired reproductive hormone, has been found to help reduce the risk of preterm birth in selected patients. It is also claimed that in women with single gestation pregnancies and a history of spontaneous preterm delivery, antenatal progesterone therapy effectively decreases the risk of recurrent preterm delivery.[4] Progesterone supplementation is beneficial in these women from 16 to 24 weeks gestation and continues until 34 weeks gestation.[4] For this purpose, progesterone can be delivered orally, intramuscularly, or as a vaginal tablet.[4] Another study suggested that pregnant women who had either a prior history of increased risk or current asymptomatic cervical insufficiency use progesterone as primary prevention for preterm labor.[5] However, progesterone was recommended as a daily supplement until the end of 34 weeks gestation,[5]. Still, other report suggested that progesterone may be useful for the maintenance of patients who had an episode of threatened preterm labor successfully treated with acute tocolytic therapy.[6]

Nifedipine is the prototype of the dihydropyridine class of calcium-channel antagonists. Originally used as a vasodilator, its structure and pharmacological effect are similar to other dihydropyridines, including amlodipine, felodipine, isradipine, and nicardipine.[7] Nifedipine inhibits the influx of extracellular calcium into cells and inhibits contraction of muscle cells.[7] In the management of preterm labor, the clinical practice guidelines recommend the use of a first-line tocolytic (i.e., beta agonist, calcium channel blocker, or NSAID) for short-term use (up to 48 hours) to allow for lung maturation.[5,7] According to one study, the number needed to treat nifedipine in cases of imminent preterm labor was 5.6 against 3.4 in the placebo group.[8] Another report showed that the number of treatments needed for nifedipine was superior to that of intramuscular progesteron.[9]

Some conflicting results were obtained from previous studies comparing nifedipine and progesterone as primary (acute)

tocolysis.[8-10] Furthermore, no tocolytic agents were chosen as first-line drugs, as it should be individually tailored.[5] Without disregarding clinical assessment, tocodynamometry can be used to accurately record uterine contraction frequency, duration, and strength.[11] Simultaneously, the cardiotocography unit is very useful for continuous fetal heart monitoring, especially during high-risk labor.[11]

Calponin is an action-binding and tropomyosin-binding cytoskeleton-associated protein. Calponin 1 was the first calponin isoform identified in differentiated smooth muscle cells and its function in regulating smooth muscle contractility.[12] Data from numerous in vitro experiments suggest that Calponin 1 functions as an inhibitory regulator of smooth muscle contractility by inhibiting actomyosin interactions. In this regulation, Calponin 1 is dissociated from the actin filament, and smooth muscle contraction is aided by Ca2+-calmodulin binding and PKC phosphorylation.[13] The timely onset of powerful uterine contractions during parturition occurs through thick and thin filament interactions, similar to other smooth muscle tissues in which calponin is one of the thin filament proteins.[14] A previous study reported that maternal serum calponin-1 level was significantly high in women who delivered within 7 days after the onset of preterm labor.[15] The threshold value of 2 ng/mL for maternal serum calponin-1 predicted delivery within 7 days with 61.1% sensitivity and 62.2 specificity (area under curve 0.658, CI 0.53–0.79). The accuracy of calponin level as a predictor of preterm delivery within 7 days was almost similar to cervical length measurements.[15]

Methods

This randomized, single-blind, interventional study was conducted at four hospitals between September and November 2016. The Dr. Hasan Sadikin General Hospital was the study center, while Cibabat Regional Hospital, Ujung Berung Regional Hospital, and R. Syamsudin SH Regional Hospital were selected as study sites due to a limited number of cases for conservative management for premature contraction were referred to the study center. The Health Research Ethics Committee of Dr. Hasan Sadikin Hospital assessed the study proposals and granted ethical clearance. Pregnant women were informed about the study and provided signed informed consent to participate. The inclusion criteria were (1) pregnancy was between 28 and 34 weeks gestation, (2) premature contraction, a condition of first episodes of painful and regular uterine contractions at least 1 time every 10 minutes and persisting for more than 30 minutes without dilatation of the cervix, and (3) evidence of cervix shortening less than 3 cm. Gestational age was determined by calculation from the date of the first day of the last menstrual period. A tocodynamometer was used to identify and record contraction in the first place. Moreover, cervical shortening was assessed by transvaginal ultrasonography.

Meanwhile, the exclusion criteria were (1) premature rupture of the membrane, (2) preeclampsia or hypertension, (3) antepartum hemorrhage, (3) polyhydramnios, (4) urogenital infection, (4) diabetes, and (5) any form of heart or kidney diseases.

Sample size calculation and statistical analysis were done, accounting for an 80% power test and 95% confidence interval; at least 13 patients were required on each arm. Assuming withdrawal and bias were reduced, an additional 30 % of study subjects were targeted. Minimum sample requirements were attained, and additional samples were added up to 22 subjects on each arm. Pregnant women were randomly assigned to either receive an oral dose of 20 mg of nifedipine three times a day or 400 mg of intra-vaginal natural progesterone once a day. The

observing physicians and investigator were blinded on one side, but not the physician who administered the medication. Allocation to treatment and laboratory results were revealed to investigators at the end of the study. The primary outcome was a reduction in the frequency and strength of contractions. Tocodynamometer measurements of contraction frequency and strength were taken before and after 4 hours of treatment, then 24 hours (day 1) and 48 hours (day 2) after treatment initiation.

The frequency was measured per 10 minutes of observation, while the strength was measured by durations of contractions (in seconds). Treatment was discontinued when active labor began, and the results were analyzed. Fetal well-being was monitored according to the hospital protocols. The secondary outcome was the change of serum calponin before and 48 hours after initial treatment. Meanwhile, calponin was measured using ELISA at Prodia, an independent, internationally accredited laboratory.

Results

As anticipated, each group included 22 pregnant women willing to participate in the survey. The characteristics for both groups were similar, as shown in the following table.

Figures 1 and 2 show how the frequency and strength of contractions changed over time. In both groups, the frequency of contractions was reduced, as seen in Figure 1. Although the baseline contraction frequency in the progesterone group was decreased, it was statistically insignificant. Cessation in frequency and strength of contraction occurred in both groups. There was no evidence of vigorous labor progress. Neither pregnant mothers nor their fetuses showed any detrimental effects.

The frequency of contractions decreased in almost identical ways in both groups. The progesterone group began and ended with a bit lower frequency. Table 2 shows the changes in each step of observation from baseline.

Reduction of contraction strength was experienced by both groups. The progesterone group began with stronger contractions, decreased significantly faster after treatment began, and finished with slightly weaker contractions. Table 2 shows the changes in each step of observation. For sequential observation, statistical analysis was performed to compare any differences between the groups.

The results show that progesterone considerably lowered the strength of contractions after 4 hours of initial treatment. It occurred since progesterone was superior in reducing both the frequency and strength of contractions.

Meanwhile, the smooth slope of contraction decrease in the nifedipine group was disrupted on day 1, as illustrated in Figure 2. To quantify the effect of treatment, another analysis was performed to compare the proportion changes in both groups. The comparison results are shown in the following table.

Reduction of frequency and strength of contraction, calculated as proportion, showed a slower reduction rate. However, the reduction of contraction strength showed some difference between the two groups, although statistically insignificant. The calponin level was then compared between baseline and day 2.

The progesterone group had an 11.32% decrease in serum calponin, while the nifedipine group had a 0.84% increase in serum calponin. It is suggested that progesterone 218 might have disrupted the pathway of uterine muscle contraction by binding with the G-receptor to prevent uterine muscle contraction.[5] This study shows that both progesterone and nifedipine had the same result in the prevention of labor until

Table 1 Characteristics of Pregnant Women

Characteristics	Intervention	
	Progesterone (n=22)	Nifedipin (n=22)
Age (year)		
<20	4	4
20-24	3	7
25-29	6	2
30-34	4	6
≥ 35	5	3
Mean (SD)	28(8.19)	27(7.39)
Range	16-44	15-41
Gestational age (week)		
28-29	10	5
29-30	0	0
30-31	3	15
31-32	3	4
32-33	1	1
33-34	5	7
Parity		
0	10	7
1-2	12	24
>2	0	1
Educational Background		
Primary School	9	13
Junior High School	2	6
Senior High School	8	12
Academy/College	3	1
Occupational status		
Working	14	20
Not Working	8	12
Cervical length (cm)		
Mean (SD)	2.72 (0.43)	2.66 (0,46)
Range	2.1-3.44	1.56-3.54

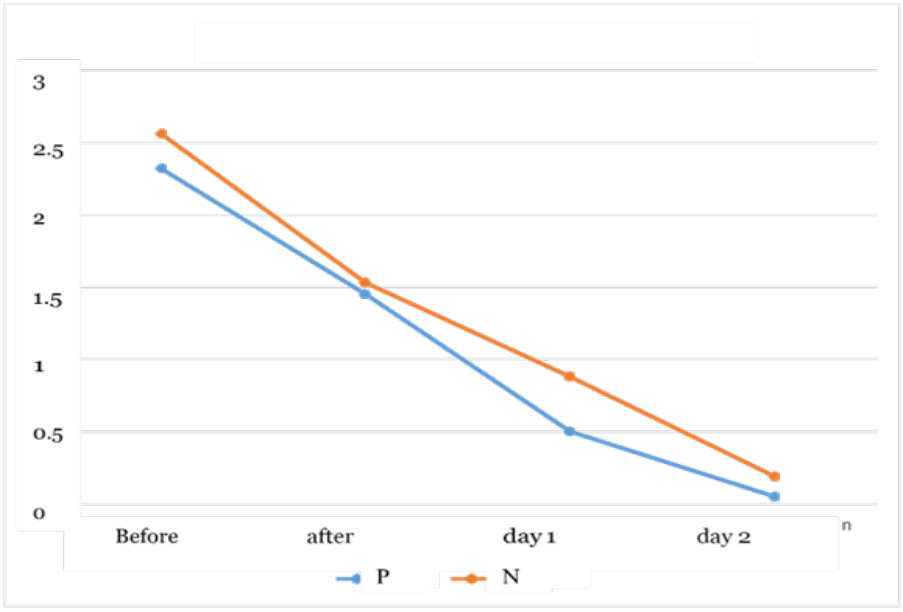


Figure 1 Reduction in Contraction Frequency

Note: P = progesterone; N = nifedipine.
frequency: number of contractions per 10 minutes.
after = after 4 hours of initial treatment,
day 1 = 24 hours after initial treatment.
day 2 = after 28 hours of initial treatment

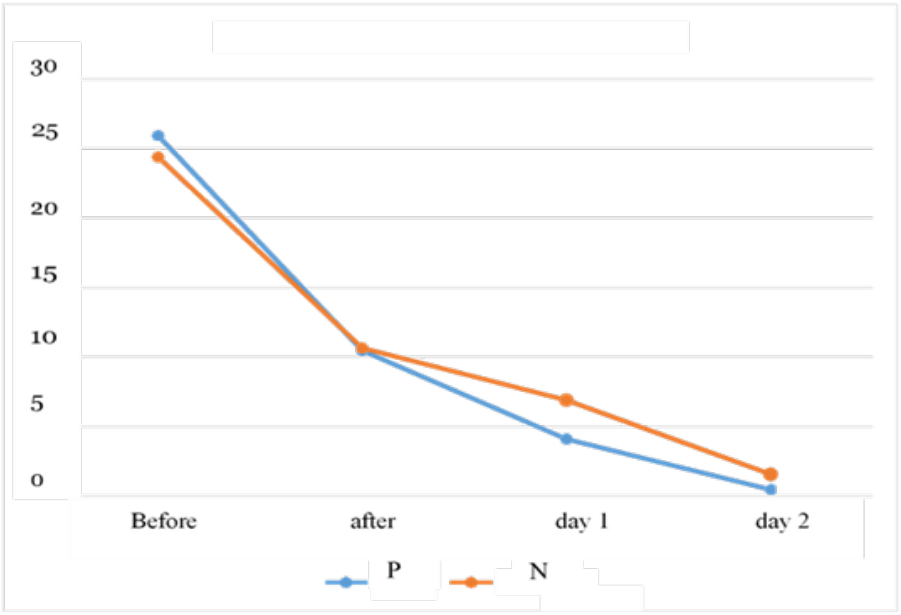


Figure 2 Reduction in contraction strength

Note: P = progesterone; N = nifedipine.
frequency: number of contractions per 10 minutes.
after = after 4 hours of initial treatment,
day 1 = 24 hours after initial treatment.
day 2 = after 28 hours of initial treatment

Table 2 Sequential Comparison of Contraction Frequency and Strength

Variable	Intervention		
	Progesterone (n=22)	Nifedipine (n=22)	p value*
Frequency of Contraction			
Baseline			
Mean (SD)	2.32 (0.48)	2.56 (0.50)	0.11
4 hours after initial treatment			
Mean (SD)	1.45 (0.74)	1.53 (0.76)	0.73
Day 1			
Mean SD)	0.50 (0.74)	0.88 (0.78)	0.10
Day 2			
Mean (SD)	0.05 (0.21)	0.19 (0.47)	0.21
Strength of Contraction			
Baseline			
Mean (SD)	25.91 (5.03)	24.38 (5.04)	0.32
4 hours after initial treatment			
Mean (SD)	10.45 (6.53)	10.63 (5.04)	0.09
Day 1			
Mean (SD)	4.09 (5.03)	6.88 (5.92)	0.09
Day 2			
Mean (SD)	0.45 (2.13)	1.56 (3.69)	0,25

Note: day 1 = after 24 hours of initial treatment.
day 2 = after 48 hours of initial treatment
p-value based on t-test

Table 3 Comparison of Changes in Contraction Frequency and Strength

Variable	Intervention		
	Progesterone (%)	Nifedipine (%)	p value**
Reduction in Frequency of Contraction*			
After 4 hours of initial treatment	37.50	40.23	0.83
Day 1 (after 24 hours of initial treatment)	65.52	42.48	0.13
Day 2 (after 48 hours of initial treatment)	90.00	78.41	0.293
Reduction in Strength of Contraction*			
After 4 hours of initial treatment	59.67	56.40	0.82
Day 1 (after 24 hours of initial treatment)	60.86	35.28	0.09
Day 2 (after 48 hours of initial treatment)	89.00	77.33	0.31

Note: * presented as proportion from previous result, p-value by t-test

Table 4 Serum Calponin Level

Serum Calponin	Intervention		
	Progesterone (n=22)	Nifedipine (n=22)	p value
Baseline			
Mean (SD)	0.53 (0.44)	0.3587 (0.15)	0.09
Day 2 (48 hours after initial treatment)			
Mean (SD)	0.47 (0.46)	0.3617 (0.18)	0.31
Reduction of serum calponin	Yes	No	
Proportion change from baseline	(-) 11.32 %	(+) 0.83 %	

48 hours after admission. The mean serum calponin level in all subjects was less than 2 ng/mL, which favors a good outcome when compared to the use of Calponin as a predictor of preterm labor.[15] Although statistically insignificant, the baseline cervical length in the calponin group was slightly longer than in the nifedipine group, which was also associated with a favorable outcomes. The inclusion criteria for this study fulfilled 4/5 of the tocolysis indications, with the exception that the mean cervical length was more significant than 2.5 cm.[5] Overall, the initial prognosis for all cases was favorable, which explains the successful management of all cases in this study.

Despite the recommendation that progesterone was used only for primary prevention and not tocolysis,[5] this study shows that 400 micrograms of intra-vaginal natural progesterone given three times a day was superior to 20 mg oral nifedipine given three times a day. As shown in Figures 1 and 2, progesterone caused a faster and more consistent decrease in frequency and strength. Progesterone has an important role in pregnancy since it helps maintain uterine stability during pregnancy and suppresses uterine contractility by binding with the G receptor.[5]

Progesterone usage was associated with a significant decrease in fetal side effects, namely, respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, neonatal jaundice, and neonatal intensive care, and maternal side effects, namely, hypotension, arrhythmia, and hypoxia, compared to other tocolitics.[5]

Moreover, calponin inhibits the contraction of smooth muscle by binding/ interacting with actin and calmodulin, which then inhibits action-activated myosin ATPase activity without affecting myosin light chain (LC20) phosphorylation.[5,13] Increased free calponin in serum would result otherwise. Table 4 shows that the mean calponin level in the nifedipine group was lower at baseline than in the progesterone group; meanwhile, calponin levels in the nifedipine group increased on day 2. Further study is needed to confirm how progesterone showed superiority in this study.

Discussion

This study shows that both progesterone and nifedipine had the same result in the prevention of labor until 48 hours after admission. The mean serum calponin level in all subjects was less than 2 ng/mL, which favors a good outcome when compared to the use of Calponin as a predictor of preterm labor.15 Although statistically insignificant, the baseline cervical length in the calponin group was slightly longer than in the nifedipine group, which was also associated

with a favorable outcomes. The inclusion criteria for this study fulfilled 4/5 of the tocolysis indications, with the exception that the mean cervical length was more significant than 2.5 cm.5 Overall, the initial prognosis for all cases was favorable, which explains the successful management of all cases in this study.

Despite the recommendation that progesterone was used only for primary prevention and not tocolysis,5 this study shows that 400 micrograms of intra-vaginal natural progesterone given three times a day was superior to 20 mg oral nifedipine given three times a day. As shown in Figures 1 and 2, progesterone caused a faster and more consistent decrease in frequency and strength. Progesterone has an important role in pregnancy since it helps maintain uterine stability during pregnancy and suppresses uterine contractility by binding with the G receptor.5

Progesterone usage was associated with a significant decrease in fetal side effects, namely, respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, neonatal jaundice, and neonatal intensive care, and maternal side effects, namely, hypotension, arrhythmia, and hypoxia, compared to other tocolitics.5

Moreover, calponin inhibits the contraction of smooth muscle by binding/ interacting with actin and calmodulin, which then inhibits action-activated myosin ATPase activity without affecting myosin light chain (LC20) phosphorylation.5,13 Increased free calponin in serum would result otherwise. Further study is needed to confirm how progesterone showed superiority in this study.

Conclusion

This study exposed that both progesterone and nifedipine were comparable in terms of successfully postponing preterm birth, with no evidence of active labor occurring until after 48 hours of initial treatment. Reducing maternal calponin 1 was significant evidence that natural progesterone was superior to nifedipine in the treatment of uncomplicated premature contraction.

Conflict Of Interest

The authors of this study declare that there is no conflict of interest.

References

1. Purisch SE, Gyamfi-Bannerman C. Epidemiology of preterm birth. Semin Perinatol. 2017;41(7):387–391.
2. Mohamed IT. Calponin 1 serum level: a biological marker for preterm labor predictability. Interventions in Gynaecology and Women's Healthcare. 2018;2(4).
3. NICE. National Institute for Health and Care Excellence. Biomarker tests to help diagnose preterm labour in women with intact membranes (DG33). In: https://www.clinicalkey.com/#!/content/nice_guidelines/65-s2.0-DG33. Accessed 18 May 2018
4. Gold Standard. Drug monograph: Progesterone. https://www.clinicalkey.com/#!/content/drug_monograph/6-s2.0-515. Accessed 19 April 2019
5. Schleußner E. The prevention, diagnosis and treatment of premature labor. Deutsches Arzteblatt international. 2013;110(13):227–236.
6. Ding M-X, Luo X, Zhang X-M, Bai B, Sun J-X, Qi H-B. Progesterone and nifedipine for maintenance tocolysis after arrested preterm labor: A systematic review and meta-analysis of randomized controlled trial. Taiwanese Journal of Obstetrics and Gynecology. 2016;55(3):399–404.
7. Gold Standard. Drug Monograph:

Nifedipine.
8. Songthamwat S, Na Nan C, Songthamwat M. Effectiveness of nifedipine in threatened preterm labor: a randomized trial. Int J Womens Health. 2018;10:317–323.
9. Haghighi L, Rashidi M, Najmi Z, et al. Comparison of intramuscular progesterone with oral nifedipine for treating threatened preterm labor: A randomized controlled trial. Med J Islam Repub Iran. 2017;31:56.
10. Chawanpaiboon S, Pimol K, Sirisomboon R. Comparison of success rate of nifedipine, progesterone, and bed rest for inhibiting uterine contraction in threatened preterm labor. Journal of Obstetrics and Gynaecology Research. 2011;37(7):787–791.
11. Miller DA. Intrapartum Fetal Evaluation. In: Gabbe SG, Niebyl JR, Simpson JL, et al., eds. Obstetrics: Normal and Problem Pregnancies. 7th ed.2017: https://www.clinicalkey.com/#!/browse/book/3-s2.0-C20130004082.
12. Doyle LA, Hornick JL. Immunohistology of Neoplasms of Soft Tissue and Bone. In: Dabbs DJ, ed. Diagnostic Immunochemistry: Theranostic and Genomic Applications 5th Ed. Pennsylvania: Elsevier; 2019:86.
13. Liu R, Jin JP. Calponin isoforms CNN1, CNN2 and CNN3: Regulators for actin cytoskeleton functions in smooth muscle and non-muscle cells. Gene. 2016;585(1):143-153.
14. Lesai L, Yong Z, Changju Z. Phosphorylation of h1 Calponin by PKC epsilon may contribute to facilitate the contraction of uterine myometrium in mice during pregnancy and labor. Reprod Biol Endocrinol. 2012;10(37).
15. Cetin O, Karaman E, Boza B, Cim N, Sahin HG. Maternal serum calponin 1 level as a biomarker for the short-term prediction of preterm birth in women with threatened preterm labor. J Maternal Fetal Neonatal Med. 2018;31(2):216–222.