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Decreased Adherence and Spontaneous Separation of Fetal Membrane Layers--Amnion and Chorion--a Possible Part of the Normal Weakening Process

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Abstract

The fetal membrane (FM) layers, amnion and chorion, are frequently noted to have varying degrees of separation following delivery. FM layers normally separate prior to rupture during *in vitro* biomechanical testing. We hypothesized that the adherence between amnion and chorion decreases prior to delivery resulting in separation of the FM layers and facilitating FM rupture.

METHODS—FM from 232 consecutively delivered patients were examined to determine the extent of spontaneous separation of the FM layers at delivery. Percent separation was determined by the weight of separated FM tissue divided by the total FM weight. Separately, the adherence between intact FM layers was determined. FM adherence was tested following term vaginal delivery (13), term unlabored cesarean section (10), and preterm delivery (6).

RESULTS—Subjects enrolled in the two studies had similar demographic and clinical characteristics. FM separation was present in 92.1% of membranes. Only 4.3% of FM delivered following spontaneous rupture of the fetal membranes (SROM) had no detectable separation. 64.7% of FM had greater than 10% separation. FM from term vaginal deliveries had significantly more separation and were less adherent than FM of term unlabored, elective cesarean section ($39.0 \pm 34.4\%$ vs $22.5 \pm 30.9\%$, $p=.046$ and 0.041 ± 0.018 N/cm vs 0.048 ± 0.019 N/cm, $p<.005$). Preterm FM had less separation and were more adherent than term FM ($9.95 \pm 17.7\%$ vs $37.5 \pm 34.4\%$ and 0.070 ± 0.040 N/cm vs 0.044 ± 0.020 N/cm; both $p<.001$).

CONCLUSIONS—Separation of the amnion from chorion at delivery is almost universal. Increased separation is associated with decreased adherence as measured *in vitro*. Increased

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separation and decreased adherence are seen both with increasing gestation and with labor suggesting both biochemical and mechanical etiologies. The data are consistent with the hypothesis that FM layer separation is part of the FM weakening process during normal parturition.

Introduction

Rupture of the fetal membranes (FM) typically follows the onset of uterine contractions; however, in up to 10% of term births, premature rupture of FM (PROM) precedes the onset of contractions. In preterm births, pPROM is the sentinel event in approximately one third of cases and is thus the proximate cause of significant neonatal mortality and morbidity.

The mechanisms by which term or preterm FM weaken and rupture are not completely understood. Previously, we have shown that term FM weaken prior to the onset of labor [1] and that weakening is not solely due to the acute mechanical forces of labor contractions, but rather the result of a biochemical process characterized by apoptosis and extracellular matrix remodeling [1,2]. Furthermore, weakening of term FM is not homogeneously distributed across the entire surface, but is more localized to the area that overlies the uterine cervix [1,3]. This physiologic “weak zone” is present prior to the onset of labor in the third trimester, as demonstrated from studies of term FM from elective, scheduled, unlabored cesarean sections as well as from spontaneous vaginal delivery (SVD) specimens [1,3]. The acute stretch forces of contractions during the normal labor process may secondarily lead to the rupture of the biochemically weakened FM.

We have observed that layers of the FM (amnion and choriondecidua) are frequently partially separated at delivery. Significant separation has frequently been a reason for elimination of specific FM from research studies, particularly biomechanical studies, by our group and others. Often this separation is in the region of the weak zone. Additionally, during *in vitro* experiments designed to explore the FM rupture process, we video-documented consistent separation of the amnion from the choriondecidua layers occurring prior to rupture [4]. In the same report we demonstrated that the work [energy] required to rupture FM pieces with fused amnion and choriondecidua is significantly greater than the sum of the work required to rupture adjacent, manually-separated pieces of individual amnion and choriondecidua [4]. Thus part of the work necessary to rupture normal, adherent FM is expended during the separation of FM layers and this separation of the FM reduces the amount of force necessary to rupture the membranes.

Meinert et al have also reported FM separation and presented specific evidence that changes in the proteoglycans, decorin and biglycan, and especially increases in the glycosaminoglycan, hyaluronan, at the interface of the amnion and choriondecidua contribute to this process [5,6]. They suggested that the changes in decorin and biglycan may result in collagen fiber disorganization and that hyaluronan may absorb water, with resultant increases in tissue pressure, causing FM separation and weakness.

Based upon these observations, we hypothesized that FM separation is a part of the FM weakening process facilitating rupture of membranes. We further hypothesized that if separation of the layers of FM were part of the normal FM weakening process, a mechanism for gradual weakening of the tissue bonds fusing the amnion and choriondecidua, like that proposed by Meinert et al [6], must exist which would decrease the adherence between the amnion and choriondecidua at the end of gestation. Physical principles dictate that separation of the FM layers should occur when mechanical shear forces exceed the adherence, as determined by these tissue bonds. Thus separation would become more likely as adherence decreases. The aims of this study were to determine the degree of separation of FM layers in normal deliveries and, secondly, to relate the degree of separation to the strength of adherence between the FM layers. To facilitate the latter, we have developed and reported an *in vitro*

testing methodology to measure the adherence between intact FM layers [7]. If our hypothesis that FM separation is a normal physiological process is correct, FM from clinically defined groups (i.e. Cesarean section delivery without labor, AROM, SROM, preterm and term) which show greater separation should show parallel decreases in adherence.

Methods

Tissue Collection

In Vivo Study- Determination of the normal degree of Separation of Amnion and Choriondecidua—This study was approved by the Institutional Review Board of the MetroHealth Medical Center. MetroHealth Medical Center is a referral center with approximately 3600 annual births, a significant proportion of which are high risk and/or premature births. Following delivery, all placentae with attached FM are routinely stored in a cold storage facility for one week. Placentae are generally placed in cold storage within 30 to 60 minutes of delivery. During two study periods of eight weeks duration, during each of the summers of 2006 and 2007, FM from all deliveries were considered for study. Two procedures were followed: 1) For deliveries occurring between 8 am to 5 pm, FM were collected shortly after delivery and examined immediately; 2) For deliveries occurring between 5 pm and 8 am, FM were collected and processed the following morning. FM delivered more than 24 hours earlier were excluded. As our objective was to study normal tissue, placentae from labored cesarean sections, with clinical chorioamnionitis, pre-eclampsia, IUGR, FM of multiples, those earmarked for pathological examination for clinical management, and those with gross placental anomalies were excluded. It is not the practice of our pathology service to routinely examine all placentas, thus histological information is not available for FM in the *in vivo* study.

In Vitro Study-Adherence Testing—To determine the force of adherence between FM amnion and choriondecidua, we utilized our previously published equipment and protocol to perform standard T-peel testing [7]. FM (n=29) were collected for these studies as follows: term vaginal delivery (n=13: SROM, n=7; AROM, n=6), term unlabored cesarean section (n=10), preterm vaginal delivery (n=5: SROM, n=3; AROM n=2) and indicated preterm unlabored cesarean section (n=1). The same exclusion criteria were used as for the separation study. In addition, FM with histological chorioamnionitis were also excluded. The FM were processed, as described below, within 20 minutes following delivery.

Quantification of Spontaneous FM Separation

FM were cut from the placental disc, washed with Hanks Balanced Salt Solution, removed of blood clots, and then laid flat with amnion side up. Membranes were visually examined for areas of obvious spontaneous separation by teams of two investigators (DK, PS, JS, KB, JN) working together. Separation was usually evident by differences in appearance and translucency between separated versus adherent areas of FM. If an obvious plane of separation was determined and confirmed by both investigators, the amnion was gently manually lifted until the amnion could no longer be lifted without the choriondecidua also being pulled up (Figure 1A). All areas where the amnion was separated from the choriondecidua were then excised. Hanks solution was gently blotted off the FM prior to weighing. For each placenta, the separated areas (amnion plus choriondecidua) were weighed together. The remaining adherent membranes were then weighed. The proportions of separated FM were reported by dividing the weight of the separated FM by the total weight of the combined (separated plus adherent) membranes.

This methodology assumes that equal surface areas of FM fragments of the same patient have equal weight. FM are known to be heterogeneous, however. Specifically, FM in the area of the weak zone are known to have decreased decidua which may decrease fragment weight [1,8].

The weak zone may also have increased hyaluronan with absorbed water which may increase fragment weight [6]. We have compared equal size FM fragments cut from the separated and non-separated regions of the same placenta and found differences in weight/surface area of less than 5% (data not shown).

Effect of Refrigeration Storage Time upon FM Separation

As not all placentae were examined immediately after birth, we determined the possible effects of delay in examination and refrigeration upon spontaneous FM separation. Six FM with varying degrees of spontaneous FM separation were obtained immediately following delivery. Gentian violet was used as a dye to mark along the line of separation between amnion and chorion. The marked FM were then placed in a refrigerator (-4°C). Placentae were re-examined by two investigators every 8 hours to assess for progression of the spontaneous FM peel front. Subsequent observation for up to 24 hours did not show progression of the line of separation between the amnion and chorion. Separately, the percentage of spontaneous FM separation was evaluated in 20 fresh FM less than one hour after delivery and compared with the extent of spontaneous FM separation in 200 refrigerated FM, one to less than 24 hours old. No difference in degree of spontaneous FM separation [fresh ($36.1 \pm 32.7\%$) versus refrigerated ($37.2 \pm 34.1\%$); $p=0.88$] was found.

Determination of Adherence between Amnion and Chorion

FM used for the adherence study were obtained fresh from the delivery room and immediately taken to the laboratory for study. The force of FM adherence was determined using our published T-peel testing protocol [7]. Briefly, the entire FM was cut from the placental disc. Membranes were gently washed with refrigerated Minimum Essential Medium with Earle's Salts (MEM: Mediatech, Manassas, Va) and all blood clots were removed. Membrane pieces were kept moist with MEM throughout the peel testing process. Once removed from the disc, FM were laid flat in MEM. A series of cuts were made starting from the placental disc edge and extending to the incision/tear line. This allowed the FM to lay completely flat. Serial secondary cuts were made along a line extending from the disc to the incision/tear line (cesarean section/vaginal delivery) to obtain tissue test pieces (4×6 cm). Additional FM sections, parallel and adjacent to this group, were cut maintaining similar orientation. Tracing paper was used to document the orientation of FM to the placental disc edge as well as the incision/tear (cesarean section/vaginal delivery) line. A 4×6 cm cork sheet template placed gently over the FM guided the placement of a 4 cm microtome blade to obtain uniform sized FM pieces. This allowed 9 to 15 test samples per FM. Location of samples was mapped on the tracing paper to maintain sample orientation and samples were stored in MEM and refrigerated until testing. These FM test strips were used to determine individual and average force of FM layer adherence for each placenta. Commercial industrial tensile testing equipment (701 Universal Test System, Com-Ten Industries, St. Petersburg, FL) was adapted to perform a standard engineering T-peel test (ASTM D1876) to measure FM adhesive force with a sensitivity of 0.05 mg. Each 4×6 cm piece was loaded in a customized clamp system on the T-peel tester where the lower clamp is held stationary while the upper clamp, attached to the load cell, is driven vertically (peel speed 25.4 cm/min) by the peel tester, peeling the amnion from the chorion (Figure 1B, 1C). Average peel force per individual sample tested was determined from the force (N) versus displacement (cm) curve. Force of adherence (N/cm) is reported as peel force per sample divided by the undeformed sample width of the tested FM fragments (4 cm). Average adherence force for each FM was determined by dividing the sum of the average forces of all samples by the number of all samples for that FM (average of 12 pieces per FM).

Clinical Data Collection and Analysis

Maternal records and the obstetric summary were reviewed for each patient. For the purpose of this study, the time of initial patient assessment by labor and delivery nursing staff in the triage room was taken as the admission time. Comparisons among groups were analyzed using ANOVA and the Student's t-test on Statview (Version 5.0.1). Differences were considered statistically significant if $p < 0.05$ (two-tail).

Results

Patient characteristics

Our objective was to study consecutive, normal FM. From a total of 770 deliveries during the two clinical study periods, 232 FM (30%) were examined. Of the 538 unexamined FM, 207 were sent to pathology for clinical management, 302 FM were not used because they were more than 24 hours post-delivery before they could be examined, and 29 were discarded due to exclusion criteria. Additional FM (29) were collected for *in vitro* analysis of FM layer adherence. The demographic and labor details for these FM were similar to those used for the *in vivo* studies of spontaneous FM separation (Table 1).

Frequency of spontaneous FM separation

The frequency distribution of spontaneous FM separation revealed a wide range of spontaneous separation among 232 FM (Figure 2). Some degree of FM separation was seen in 92.1% of membranes. Of these, 64.7% exhibited greater than 10% separation of the amnion from the choriodecidua (Figure 2). No separation was detectable in 7.9% of the examined FM, and in only 4.3% of FM delivered following SROM. When separation was detected, it always involved some part of the rupture tear line. There was no association between percent of spontaneous FM separation or FM layer adherence with fetal gender, maternal race, maternal tobacco use, antibiotic use during labor, documented infection(s) during pregnancy or labor, or oxytocin use (Table 2).

Labor characteristics and FM layer separation and adherence

FM from term vaginal delivery following all modes of rupture (SROM and AROM) had significantly more spontaneous separation than FM from term unlabored, elective cesarean section ($p = 0.046$; Figure 3A, Table 2). *In vitro* analysis of adherence was consistent with this finding. That is, FM from term vaginal delivery following all modes of rupture had significantly lower adherence compared to FM from term unlabored, elective cesarean section ($p = 0.005$; Figure 3B, Table 2). This suggests that labor decreases adherence facilitating FM layer separation.

FM from term SVD following SROM had significantly more separation than FM from term SVD following AROM ($p = 0.046$) or from term unlabored, elective cesarean section ($p = 0.008$; Figure 4A, Table 2). Consistently, FM from term SVD following SROM had significantly lower adherence compared to FM from term unlabored, elective cesarean section ($p = 0.015$; Figure 4B, Table 2). The association between FM separation, and admission (to the hospital) to delivery time, was also assessed. Patients with SVD following SROM, had a shorter admission to delivery time than those from SVD following AROM, (496 ± 303 versus 608 ± 375 minutes; $p = 0.029$.) Thus, deliveries requiring the least intervention showed less adherence and more separation of the FM layers at birth. They also proceeded more rapidly.

Effect of gestational length on FM separation and adherence

Preterm FM had significantly less separation than term FM for all modes of delivery ($p < 0.001$; Figure 5A, Table 2). Additionally, *in vitro* testing demonstrated that preterm FM had

significantly greater adherence compared to term FM for all modes of delivery ($p < 0.001$; Figure 5B, Table 2). The differences in percentage of separation and adherence between preterm and term fetal membranes are much greater than the differences due to labor suggesting that changes in gestational length have a larger effect (Table 2).

Discussion

The key finding of this study is that some degree of spontaneous separation of FM layers (amnion and chorion) at the time of delivery is nearly universal. Further, increased separation, coupled with decreased adherence, is associated with both labor and increased gestational length, suggesting that the process has both mechanical and maturational (presumably biochemical) etiologies. Finally, greater spontaneous FM separation in association with less adherence is seen more frequently with normal birthing processes that do not require intervention (i.e., spontaneous vaginal delivery, spontaneous rupture of membranes, and term delivery). Together, these studies support the concept that FM layer separation is part of the FM weakening process during normal parturition.

There is limited medical literature focusing upon prenatal FM layer separation as documented by ultrasound. The amnion and chorion are normally separate prior to 14 weeks gestation and fuse between 14–16 weeks gestation. Separation of amnion from chorion after 16 weeks gestation is associated with adverse perinatal outcomes: including PROM, chorioamnionitis, preterm birth [9], fetal chromosomal abnormalities [10,11], amniotic bands, umbilical cord strangulation and fetal death [12,13]. Prenatal FM separation is common after invasive procedures, occurring in up to 25% of amniocentesis procedures and 12–47% of fetal surgeries [9,12,13]. Total (100%) FM separation is associated with extremely adverse perinatal outcomes [10,14]. This association between FM separation and adverse perinatal outcomes emphasizes the importance of understanding the mechanism underlying spontaneous FM separation in both normal and complicated pregnancies.

While our previous work, along with that of others, has suggested that FM layer separation may be a normal part of the FM rupture process, the steps leading to FM layer separation are not clearly defined [4–6,15,16]. Current understanding of the FM weakening and rupture process suggests that FM weaken as a result of a programmed biochemical process associated with cellular apoptosis, extra-cellular matrix remodeling and degradation [3,8,16,17]. Additional findings demonstrate that the FM weakening process initiates in the late third trimester and uterine contractions during labor likely facilitate further weakening and membrane rupture [1]. In the present study, the observation that some spontaneous separation of FM layers occurs even in term unlabored, elective Cesarean deliveries suggests that spontaneous FM separation is due, at least in part, to biochemical remodeling of the amnion-chorion interface near the end of gestation. Comparison of separation and adherence of preterm FM versus term FM further supports this concept as spontaneous FM separation was significantly more frequent and extensive in term FM (≥ 37 weeks) compared to preterm FM (< 37 weeks), independent of duration of labor. Furthermore, FM layer adherence decreased with increasing gestation.

Data from other groups also suggest biochemical changes at the amnion-chorion interface promote FM separation and weakness. Meinert et al [5,6] and Helmig et al [18] have reported changes in the proteoglycans decorin and biglycan, in conjunction with large increases in hyaluronan at this interface during late gestation. Decorin links fibrillar collagen and organizes it into coordinated bundles. Biglycan has the opposite effect by interfering with decorin organization [19,20]. Meinert et al reported increases in biglycan in the para-cervical weak zone of FM at end gestation. Furthermore, Meinert et al demonstrated a threefold increase in hyaluronan over the last week of gestation, localized to the interface between the amnion

and choriodecidua in the para-cervical weak zone [6]. Hyaluronan is known to absorb water thereby increasing tissue pressure in the extra-cellular matrix [21]. They suggest that the increased tissue pressure coupled with collagen fiber disorganization results in FM separation.

Although we have previously reported that the FM weakening process is primarily mediated by biochemical remodeling that occurs prior to labor rather than during labor itself [1–3], it is clear from the data above that mechanical forces of labor also affect fetal membrane adhesiveness and membrane separation. Term FM from vaginal deliveries show less adherence and more separation than term FM obtained after cesarean section without labor. Although the differences in both separation and adherence due to labor are much less than those due to gestation, to the extent that mechanical forces of labor cause separation of the FM layers, they must be considered part of the weakening process. Furthermore, FM separation is consistently seen along the tear line, in the region of the normal weak zone. It presumably exacerbates the weakening due to biochemical remodeling in this region.

In conclusion, we have demonstrated that some degree of spontaneous separation of FM layers is nearly universal following delivery, and that the propensity for separation can be quantified by determining the force of adherence using a novel methodology of FM T-Peel testing. Furthermore, we now suggest that the programmed biochemically-mediated process of FM weakening and rupture includes both weakening of the FM layers [1,3] and, in addition, decreased adherence resulting in the separation of the FM layers. The contractions of labor augment the decreases in adherence, as well as FM separation, and thereby also contribute to the FM weakening. We speculate that FM layer separation is a part of the normal parturition process and facilitates rupture of the FM.

Acknowledgments

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Abbreviations

FM	Membranes
ROM	Rupture of Membranes
PROM	Premature Rupture of Membranes
SROM	Spontaneous Rupture of Membranes
AROM	Artificial Rupture of Membranes
SVD	Spontaneous Vaginal Delivery

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Figure 1. A. Determination of Percent Spontaneous FM Separation; B. FM in T-peel adherence testing apparatus

Amnion and choriodecidua being peeled apart between the clamp jaws (1= force sensing load cell, 2 = upper clamp pulling amnion vertically upwards, 3 = amnion peeling from choriodecidua, 4 = horizontal platform of the lower clamp supporting unpeeled FM floating over cold saline, 5 = lower clamp holding choriodecidua. (Figure 1B borrowed from reference ⁷ with permission); C. FM in T-peel testing apparatus (detail).

Figure 2. Distribution of Percent Spontaneous Separation in 232 FM

The number of FM in which the percentage of separation fell within each percentage decile are shown. Percentage separation was determined by the weight of separated areas divided by the total weight of separated plus adherent areas of each FM as described in Methods.

Figure 3. Effect of Labor upon Spontaneous FM Separation and Adherence

A FM from vaginal deliveries had significantly more spontaneous FM separation compared to those from unlabored, elective cesarean section (* $p=0.046$). **B.** FM from vaginal deliveries had significantly lower average forces of FM layer adherence compared to FM from unlabored, elective cesarean section deliveries (* $p=0.005$). Abbreviations: SVD, vaginal delivery. Data shown as mean $\pm$ SEM.

Figure 4. Effect of Mode of Delivery on Spontaneous FM Separation and Adherence

A Measurements of degree of FM separation demonstrated a clear gradient across modes of delivery: C/S < AROM SVD < SROM SVD. (C/S vs SROM SVD, * $p=0.008$; AROM SVD vs SROM SVD, * $p=0.046$). **B.** Vaginally delivered FM with both SROM and AROM had significantly lower average forces of adherence compared to FM from unlabored, elective C/S deliveries (SROM SVD vs. C/S, $p=0.015$; AROM SVD vs. C/S, * $p=0.02$). Abbreviations: C/S, cesarean section; AROM, artificial rupture of membranes; SVD, vaginal delivery; SROM, spontaneous rupture of membranes. Data shown as mean $\pm$ SEM.

Figure 5. Effect of Gestational Length upon FM Separation and Adherence

A Effect of gestational age on spontaneous FM separation. Premature FM (<37 weeks) had significantly less spontaneous FM separation compared to term FM (≥ 37 weeks) for all modes of delivery ($p < 0.001$; premature, $n=28$; term, $n=204$). Data shown as mean $\pm$ SEM. **B.** Effect of gestational age on average force of FM layer adherence. Adherence of premature FM (<37 weeks) was significantly greater than adherence of term FM (≥ 37 weeks) for all modes of delivery ($p < 0.001$; premature FM, $n=6$; term FM, $n=24$). Data shown as mean $\pm$ SEM.

Table 1

Patient characteristics.

Patient Characteristic	Spontaneous FM Separation	FM Adherence
Total Number of Subjects	232	29
Maternal Age	25.3 ± 6.3 years	23.8 ± 5.0 years
Gravida (median)	3	3
SVD (%)	210 (91)	18 (62)
Cesarean Section (%)	22 (9)	11 (38)
Duration of ROM (min)	409 ± 442 min	413 ± 293 min
Duration of Uterine Contractions	667 ± 452 min	562 ± 274 min
Duration of Maternal Admission to L&D until Birth	534 ± 357 min	539 ± 289 min
Birth Weight	3214 ± 499 gm	3092 ± 463 gm
Term Deliveries (%)	204 (87.9)	23 (79)
Premature Deliveries (%)	28 (12.1)	6 (21)
Term Gestational Age	39.3 ± 1.0 weeks	39.1 ± 1.0 weeks
Premature Gestational Age	35.0 ± 3.1 weeks	34.9 ± 1.7 weeks
African Americans (%)	102 (44)	14 (48.3)
Whites (%)	78 (34)	9 (31)
Hispanics (%)	41 (17)	4 (14)

Data shown as mean ± SD. Abbreviations: SVD, spontaneous vaginal delivery; C/S, cesarean section; ROM, rupture of membranes; L&D, labor and delivery unit.

Table 2

Clinical factors associated with percentage spontaneous FM separation and FM adherence.

VARIABLE	SPONTANEOUS SEPARATION STUDY			ADHERENCE STUDY		
	n = 232	% Spontaneous Separation	P-value	n = 29	Average Force of Adherence (N/cm)	P-value
Gender	Male (110) Female (111)	33.4 ± 35.0 38.6 ± 32.3	0.22	Male (15) Female (14)	0.050 ± 0.15 0.046 ± 0.18	0.49
Tobacco Use	Yes (68) No (155)	33.7 ± 33.6 38.6 ± 34.0	0.32	Yes (5) No (24)	0.045 ± 0.012 0.048 ± 0.017	0.63
Antibiotics (labor)	Yes (63) No (144)	31.8 ± 32.4 37.7 ± 33.2	0.24	Yes (4) No (13)	0.046 ± 0.022 0.045 ± 0.014	0.93
Any Infection(s)	Yes (126) No (95)	38.1 ± 33.7 35.7 ± 34.2	0.61	Yes (14) No (15)	0.052 ± 0.019 0.045 ± 0.013	0.29
Race	African American (100) All Others (121)	38.3 ± 34.6 36.1 ± 33.3	0.63	African American (14) All Others (15)	0.047 ± 0.015 0.034 ± 0.012	> 0.05
Oxytocin	Yes (113) No (108)	33.4 ± 32.2 40.9 ± 35.2	0.09	Yes (9) No (8)	0.043 ± 0.013 0.048 ± 0.018	0.57
Term SROM vs. AROM	SROM (59) AROM (126)	46.3 ± 37.3 35.6 ± 32.6	0.046	SROM (7) AROM (6)	0.040 ± 0.020 0.040 ± 0.017	0.85
Term SROM vs. C/S	SROM (59) C/S (19)	46.3 ± 37.3 22.5 ± 30.9	0.008	SROM (7) C/S (10)	0.040 ± 0.020 0.048 ± 0.019	0.015
Term AROM vs C/S	AROM (126) C/S (19)	35.6 ± 32.6 22.5 ± 30.9	0.118	AROM (6) C/S (10)	0.041 ± 0.017 0.048 ± 0.019	0.02
Term C/S vs. SVD	C/S (19) SVD (185)	22.5 ± 30.9 39.0 ± 34.4	0.046	C/S (10) SVD (13)	0.048 ± 0.019 0.041 ± 0.018	0.005
Premature FM vs. Term FM	Premature (28) Term (204)	10.0 ± 17.7 37.5 ± 34.4	<0.001	Premature (6) Term (23)	0.070 ± 0.040 0.044 ± 0.020	<0.001

Data shown as mean ± SD. Abbreviations: SROM, spontaneous rupture of membranes; AROM, artificial rupture of membranes; C/S, cesarean section; SVD, spontaneous vaginal delivery.