Published in final edited form as:

Reprod Biol. 2015 June ; 15(2): 79–85. doi:10.1016/j.repbio.2015.02.001.

Serum biomarkers may help predict successful misoprostol management of early pregnancy failure

Courtney A. Schreiber[a,*], **Sarah J. Ratcliffe**[b], **Kelly E. Quinley**[c], **Carrie Miller**[d], **Mary D. Sammel**[b]

[a]Penn Family Planning and Pregnancy Loss Center, Penn Medicine, University of Pennsylvania, Perelman School of Medicine, 1000 Courtyard, 3400 Spruce Street, Philadelphia, PA 19104, United States

[b]Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Perelman School of Medicine, United States

[c]Highland General Hospital | Alameda County Health System, United States

[d]Perelman School of Medicine, University of Pennsylvania, United States

Abstract

In order to simplify management of early pregnancy loss, our goal was to elucidate predictors of successful medical management of miscarriage with a single dose of misoprostol. In this secondary analysis of data from a multicenter randomized controlled trial, candidate biomarkers were compared between 49 women with missed abortion who succeeded in passing their pregnancy with a single dose of misoprostol and 46 women who did not pass their pregnancy with a misoprostol single dose. We computed the precision of trophoblastic protein and hormone concentrations to discriminate between women who succeed or fail single dose misoprostol management. We also included demographic factors in our analyses. We found overlap in the concentrations of the individual markers between women who succeeded and failed single-dose misoprostol. However, hCG levels ≥4000 mIU/mL and ADAM-12 levels ≥2500 pg/mL were independently associated with complete uterine expulsion after one dose of misoprostol in our population. A multivariable logistic model for success included non-Hispanic ethnicity and parity <2 in addition to hCG ≥4000 mIU/mL and ADAM-12 ≥2500 pg/mL and had an area under the receiver operating characteristic (ROC) of 0.81 (95% confidence interval: 72–90%). Categorizing women with a predicted probability of ≥0.65 resulted in a sensitivity of 75.0%, specificity 77.1% and positive predictive value of 81.8%. While preliminary, our data suggest that serum biomarkers, especially when combined with demographic characteristics, may be helpful in guiding patient decision-making regarding the management of early pregnancy failure (EPF). Further study is warranted.

Keywords

Biomarkers; Pregnancy; Miscarriage; Early pregnancy failure; Misoprostol

[*]*Corresponding author*. Tel.: +1 215 615 6531; fax: +1 215 615 5319. schreibe@upenn.edu (C.A. Schreiber).

1. Introduction

Misoprostol is a safe, convenient, and acceptable medication for the treatment of early pregnancy failure (EPF; pregnancy failure in the first trimester), but complete uterine evacuation is only achieved in 71% of women after a single dose of 800 μg vaginal misoprostol [1]. While surgical management (uterine aspiration) is more efficacious (98–99% success [1]), non-surgical options are important to improve access and individualize patient care. EPF is often classified as anembryonic gestation and embryonic/fetal demise and incomplete/inevitable abortion. Established predictors of success and failure among those women who use misoprostol include a greater success rate with incomplete/inevitable abortion when compared with missed abortion [1-4].

The current standard of care for misoprostol management of EPF was established by a landmark randomized controlled trial [1]. Success rates among the population in this study were 71% after one dose of misoprostol, and 84% (95% confidence interval [CI] 81%, 87%) after a second dose given three days later [1]. Studies show that misoprostol is highly effective for incomplete/inevitable abortion (≥90%) [5-8], but much less effective for anembryonic gestation and embryonic/fetal demise. Morbidity and cost could be decreased if we could better predict which women with missed abortion are likely to have a successful uterine evacuation with one dose of misoprostol [9,10]. Proteins that are derived from the trophoblast and secreted into maternal circulation are differentially expressed in ectopic as compared with intrauterine pregnancies [11,12]. These proteins and others are candidate biomarkers to distinguish between the pregnancies that may be more “resistant” to expulsion and those that will be expelled with one dose of misoprostol. The proteins and hormones we chose to investigate are some of the many makers of the “invasiveness” of trophoblastic tissue. Most women undergo phlebotomy as a part of their work up for EPF, so additional testing is feasible if it would help triage women toward a management strategy with better outcomes. In this study, we tested the hypothesis that levels of trophoblast-derived proteins could identify women who achieved uterine expulsion with one misoprostol dose from those who did not successfully expel the products of conception with one dose. We also examine clinical and demographic characteristics associated with expulsion to single-dose misoprostol in our population.

2. Materials and methods

Our study was approved by the University of Pennsylvania Institutional Review Board. We conducted a sub-analysis of the data and serum collected in the misoprostol for the management of early pregnancy failure (MEPF). The MEPF study [1] was a randomized, controlled, multicenter trial that tested the efficacy, safety and acceptability of misoprostol versus surgical management in treating early pregnancy failure. The study was a multicenter trial conducted from 2002 to 2004 where participating medical centers included the University of Pennsylvania, University of Pittsburgh, Columbia University and the University of Miami. The results of the primary study have been published. Briefly, in the MEPF trial, women presenting with first trimester pregnancy failure (anembryonic pregnancy, embryonic demise, incomplete abortion and inevitable abortion) were randomized to medication uterine evacuation with an 800 μg dose of vaginal misoprostol or

surgical uterine evacuation with vacuum aspiration. The medication-treated patients received four 200 μg tablets of misoprostol (Cytotec) into the posterior fornix of the vagina on day 1. They returned on day 3, and if sonographic evaluation demonstrated incomplete expulsion of products of conception, participants were given a second 800 μg vaginal dose of misoprostol and then returned on day 8 of the study for another evaluation. If the sonographic and clinical evaluation were consistent with complete expulsion of the products of conception at that time, women were contacted at 30 days for a final evaluation.

Serum samples from participants at all sites had been stored at the University of Pennsylvania at −62.2 °C. For the analysis presented here, we included all participants for whom complete records could be obtained from the MEPF database, and if there was sufficient residual banked serum to run analyses quantifying trophoblastic proteins including (vide infra). We included women who presented with anembryonic pregnancy or embryonic/fetal demise, and who had been randomized to misoprostol treatment. We excluded participants who presented with incomplete or inevitable abortion because misoprostol is highly effective for those diagnoses [9-11].

The MEPF study database was used to collect patient demographics, pregnancy-failure type, obstetrical history, clinical symptoms, and beta-hCG levels collected on days 1, 3, 8, and 15 of the original trial. In addition to hCG, the candidate markers we chose were selected based upon a thorough literature review. We chose proteins and hormones that are involved with the implantation process either from the maternal side or the pregnancy itself. Assays were conducted in the Basic Science Research Building at the Perelman school of Medicine. The following laboratory analyses were used to measure trophoblastic protein and pregnancy hormone levels in maternal sera:

- activin A (UCN Life Science Inc., Wuhan, China) was assayed using Quantikine Immunoassay kits. The minimum detectable limit for activin A was 1.25 mg/dL;
- ADAM-12 (Antibodiesonline, US Biologicals, Salem, MA, USA) protein was assayed using disintegrin and metalloprotease 12 (ADAM-12) ELISA kits. The minimum detectable limit for ADAM-12 was 24 pg/mL;
- human placental lactogen (HPL; ALPCO, Salem, NH, USA) was assayed using HPL ELISA kits. The minimum detectable limit for HPL was 1.25 mg/L;
- glycodelin (Cosmo Bio, Carlsbad, CA, USA) were assayed using Cusabio glycodelin ELISA kits. The minimum detectable limit for glycodelin was 0.78 ng/mL;
- progesterone (P4) and estradiol (E2) were analyzed using the Siemens Immulite 2000 by solid-phase, competitive binding chemiluminescent enzyme immunoassays (Siemens, Munich, Germany). The minimum detectable limits for P4 and E2 were 0.1 ng/mL and 15 pg/mL, respectively.

Values below detection thresholds were given a value of zero in analyses. All ELISA samples were run in duplicate and the values were averaged if not disparate. Beta-hCG values collected and measured during original MEPF trial were incorporated. For the statistical analyses, baseline characteristics of the groups were compared using Student’s t-

test for continuous variables and Chi-square or Fisher's exact test for categorical variables. Marker distributions were graphed by success or failure and assessed for normality. Visual inspection of the association between each marker and successful uterine evacuation with one misoprostol dose was evaluated. Lowess graphs [13] of the association between markers and probability of success of single dose misoprostol were used to assess linearity assumptions, and potential inflection, or cut-points for categorization of the markers. The area under the receiver operating characteristic curves (AUC) was calculated to assess the predictive performance or discriminatory ability of each marker. Logistic regression was used to determine the relationship between markers, demographic and clinical characteristics and success. The best predictive model was based on changes in the c-statistic as each variable was added to the model [14]. Variables with p values ≤0.2 were included in the model. The sensitivity and specificity, positive and negative predicted values were calculated for the optimal cut-point.

3. Results

We had sufficient banked serum and complete records from 95 women (out of a possible 491 who received misoprostol in the MEPF trial). The demographic and clinical characteristics for the 95 subjects included here were similar to the larger MEPF study population with the exception of parity: our sample had a significantly lower number of multiparous women ($p < 0.001$). Forty-nine women in our sample experienced successful uterine evacuation with a single dose of misoprostol, and 46 failed uterine evacuation with a single dose of misoprostol, requiring a second dose of misoprostol or vacuum aspiration for completion. Women with lower parity were more likely to have successful uterine evacuation with one dose of misoprostol ($p = 0.010$). In our sample, women of Hispanic ethnicity were less likely to succeed with one dose of misoprostol ($p = 0.009$). The success and failure groups were otherwise demographically similar, as shown in Table 1.

Concentrations of the trophoblastic proteins and pregnancy-related hormones are depicted in Fig. 1. A great deal of overlap is seen in the frequency distribution of individual biomarkers among single-dose successes and failures. In addition, non-linear associations were suggested by lowess graphs (data not shown), so biomarker levels were categorized based on empiric assessment of the graphs to optimize discrimination. The individual ROC curves for each marker also demonstrated poor performance as predictors of success or failure. Fig. 2 illustrates the predictive performance of the marker ADAM-12 in its original continuous form and as a grouped variable using a cut-off of ≥2500 pg/mL. The area under the Receiver-Operator Characteristic curves are similar. Comparing the biomarker groups between the successes and failures, ADAM-12 ($p = 0.032$), HPL ($p = 0.059$) and hCG ($p = 0.061$) were or approached statistical significance (Table 2). We therefore built a predictive model for success using a combination of the best performing clinical and serum variables along with important clinical characteristics. The final multivariable regression model included ethnicity, low parity, elevated hCG above 4000 mIU/mL, ADAM-12 above 2500 pg/mL, and obesity. As shown in Table 3, women of Hispanic ethnicity and parity >2 had a lower odds of successful pregnancy expulsion, while hCG ≥4000 mIU/mL and ADAM-12 ≥2500 pg/mL were independently associated with higher odds of success. Ranking our sample based on the predictive probability of successful uterine evacuation performed well,

with an area under the ROC curve of 0.81; categorizing women with a predicted probability of ≥0.65 resulted in a sensitivity of 75.0%, specificity 77.1% and positive predictive value of 81.8%. The highest predicted probability of success was 93.2% for non-Hispanic, nulliparous, non-obese women with elevated hCG above 4000 mIU/mL and ADAM-12 above 2500 pg/mL. The lowest predicted probability of success was 5.0% for Hispanic, parity ≥2, obese women with hCG below 4000 and ADAM-12 below 2500. Assuming that ADAM-12 levels may not be available in the clinical care setting, the area under the ROC curve for a model without ADAM-12 was 0.77. Using a cut-point of predicted probability of 0.55 or greater resulted in a sensitivity of 63.3%, specificity of 82.6% and positive predictive value of 79.5% for non-Hispanic, nulliparous, non-obese women with elevated hCG above 4000. Using hCG alone, the area under the ROC was 0.61 with a positive predictive of 60.9%, sensitivity of 57.1% and specificity of 60.9%.

4. Discussion

Medical management of early pregnancy failure is an acceptable and safe option for women seeking treatment for miscarriage. However, success rates are highly variable between subtypes of abortion. We sought to determine if implantation-related proteins and hormones might be associated with successful medical management of missed abortion using a single dose of misoprostol. In our sample of 95 women, we found that ADAM-12 above or equal to 2500 pg/mL and hCG above or equal to 4000 mIU/mL were both associated with increased likelihood of successful uterine evacuation with one dose of misoprostol. We also found nulliparity, non-Hispanic ethnicity, and non-obesity were qualities associated with success. Our findings support the notion that disruptions in the implantation process may mediate early pregnancy failure. ADAM-12, which has both an adhesion and protease domain, has a secreted form which is expressed in placenta, and potently provokes myogenesis. In first-trimester placental tissue, ADAM-12 is localized to the cytotrophoblasts as well as the apical side of the syncytiotrophoblast [15]. Given its localization and role in cell-fusion in other tissues, it has been postulated to play a role in syncytial fusion in the trophoblast [15]. Human CG is produced by syncytiotrophoblast trophoblasts. The biology behind the high levels of these molecules among women who are more likely to have successful uterine evacuation with prostaglandins is unknown, but may be related to the process of implantation, decidualization, and uterine sensitivity of prostaglandins.

Previously published clinical predictors of treatment success with misoprostol for EPF include lower abdominal pain, vaginal bleeding within 24 h of presentation, and nulliparity [3]. Since evidence of expulsion-in-progress (incomplete, inevitable) is the best clinical predictor of misoprostol success [3,8], and women who are asymptomatic at the time of EPF diagnosis are much less likely to experience a successful uterine evacuation with misoprostol. However, this is the first investigation of the role that trophoblastic biomarkers might play in the adherence of an abnormal pregnancy sac to the uterine wall.

Our exploratory analysis has limitations. There are many protein and cytokines that have been preliminarily implicated in EPF. Prolactin and inflammatory cytokines have studied at the level of the deciduas as opposed to the maternal serum as preformed in the current study [16,17]. Our goal, however, was to preliminarily investigate markers readily identifiable in

the serum that could aid women in choosing the appropriate management of their EPF before treatment. Whether or not tissue concentrations are reflective of serum concentrations (and vice versa) is unknown. In addition, the inflammatory response could very well be the host's reaction to the failed pregnancy as opposed to casual of the pregnancy loss. Ours was not a planned sub-study, so the sample size and power were limited by number of participants with sufficient banked serum to perform the protein assays. It is possible that a larger, more generalizable sample would produce different results. However, our sample, overall, was similar to the sample in the parent MEPF trial. The only computed difference between the study populations was that ours was of lower parity. However, both studies showed that low parity was associated with increased likelihood of expulsion to prostaglandins. Additionally, each biomarker was categorized based on empiric evaluation of the data. As such, the thresholds depicted here should not be considered as a gold standard and require additional validation.

The disparate success rates depending upon the type of spontaneous abortion require that we identify novel ways to tailor patient care: clearly, not all miscarriage patients are alike. Our data add to the existing literature that shows that clinical characteristics are useful to help guide patients toward or away from medical management of early pregnancy failure. While biomarkers are most commonly used as a potential marker for a drug response [18], here we demonstrate that trophoblastic proteins may improve our ability to make appropriate management choices in anticipation of treatment. It is also possible that the differential concentrations of these proteins could inform us about the pathophysiology of EPF. Further investigation along these lines is required.

Acknowledgments

This study was funded in part by an anonymous foundation, and the authors are very grateful for this support. The authors would like to thank the Eunice Kennedy Shriver National Institute of Child Health & Human Development for allowing us to utilize the dataset as well as the biospecimens. The authors would like to thank Dr. Kurt Barnhart for his review of this manuscript.

REFERENCES

[1]. Zhang J, Gilles GM, Barnhard K, Creinin MD, Westhoff C, Frederick MM. A comparison of medical management with misoprostol and surgical management for early pregnancy failure. N Engl J Med 2005;353(8):761–9. [PubMed: 16120856]

[2]. Jurkovic D Modern management of miscarriage: is there a place for non-surgical treatment? Ultrasound Obstet Gynecol 1998;11(3):161–3. [PubMed: 9589136]

[3]. Chen BA, Creinin MD. Contemporary management of early pregnancy failure. Clin Obstet Gynecol 2007;50(1):67–88. [PubMed: 17304025]

[4]. Creinin MD, Huang X, Westhoff C, Barnhart K, Gilles JM, Zhang J. Factors related to successful misoprostol treatment for early pregnancy failure. Obstet Gynecol 2006;107(4):901–7. [PubMed: 16582130]

[5]. Paritakul P, Phupong V. Comparative study between oral and sublingual 600 microg misoprostol for the treatment of incomplete abortion. J Obstet Gynaecol Res 2010;36(5):978–83. [PubMed: 20846257]

[6]. Dabash R, Ramadan MC, Darwish E, Hassanein N, Blum J, Winikoff B. A randomized controlled trial of 400-μg sublingual misoprostol versus manual vacuum aspiration for the treatment of incomplete abortion in two Egyptian hospitals. Int J Gynaecol Obstet 2010;111(2):131–5. [PubMed: 20801444]

[7]. Diop A, Raghavan S, Rakotovao JP, Comendant R, Blumenthal PD, Winikoff B. Two routes of administration for misoprostol in the treatment of incomplete abortion: a randomized clinical trial. Contraception 2009;79(6):456–62. [PubMed: 19442782]

[8]. Neilson JP, Gyte GM, Hickey M, Vazquez JC, Dou L. Medical treatments for incomplete miscarriage (less than 24 weeks). Cochrane Database Syst Rev 2010;20(1):CD007223.

[9]. Harwood B, Nansel T. Quality of life and acceptability of medical versus surgical management of early pregnancy failure. BJOG 2008;115(4):501–8. [PubMed: 18271887]

[10]. Rausch M, Lorch S, Chung K, Frederick M, Zhang J, Barnhart K. A cost-effectiveness analysis of surgical versus medical management of early pregnancy loss. Fertil Steril 2012;97 (2):355–60. [PubMed: 22192348]

[11]. Rausch ME, Sammel MD, Takacs P, Chung K, Shaunik A, Barnhart KT. Development of a multiple marker test for ectopic pregnancy. Obstet Gynecol 2011;117(3):573–82. [PubMed: 21343760]

[12]. Segal S, Gor H, Correa N, Mercado R, Veenstra K, Rivnay B. Inhibin A: marker for diagnosis of ectopic and early abnormal pregnancies. Reprod Biomed Online 2008;17 (6):789–94. [PubMed: 19079962]

[13]. Cleveland WS. Robust locally weighted regression and smoothing scatterplots. JASA 1979;74(368):829–36.

[14]. Harrell F Jr Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. New York: Springer; 2001.

[15]. Huppertz B, Bartz C, Kokozidou M. Trophoblast fusion: fusogenic proteins, syncytins and ADAMs, and other prerequisites for syncytial fusion. Micron 2006;37(6):509–17. [PubMed: 16497505]

[16]. Garzia E1, Clauser R, Persani L, Borgato S, Bulfamante G, Avagliano L, et al. Prolactin and proinflammatory cytokine expression at the fetomaternal interface in first trimester miscarriage. Fertil Steril 2013;100(1):108–15. e1–2. [PubMed: 23541403]

[17]. Salker M1, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. PLoS ONE 2010;5(4):e10287. [PubMed: 20422017]

[18]. Hall JA, Brown R, Paul J. An exploration into study design for biomarker identification: issues and recommendations. Cancer Genomics Proteomics 2007;4(3):111–9, http://cgp.iiarjournals.org/content/4/3/111.long. [PubMed: 17878515]

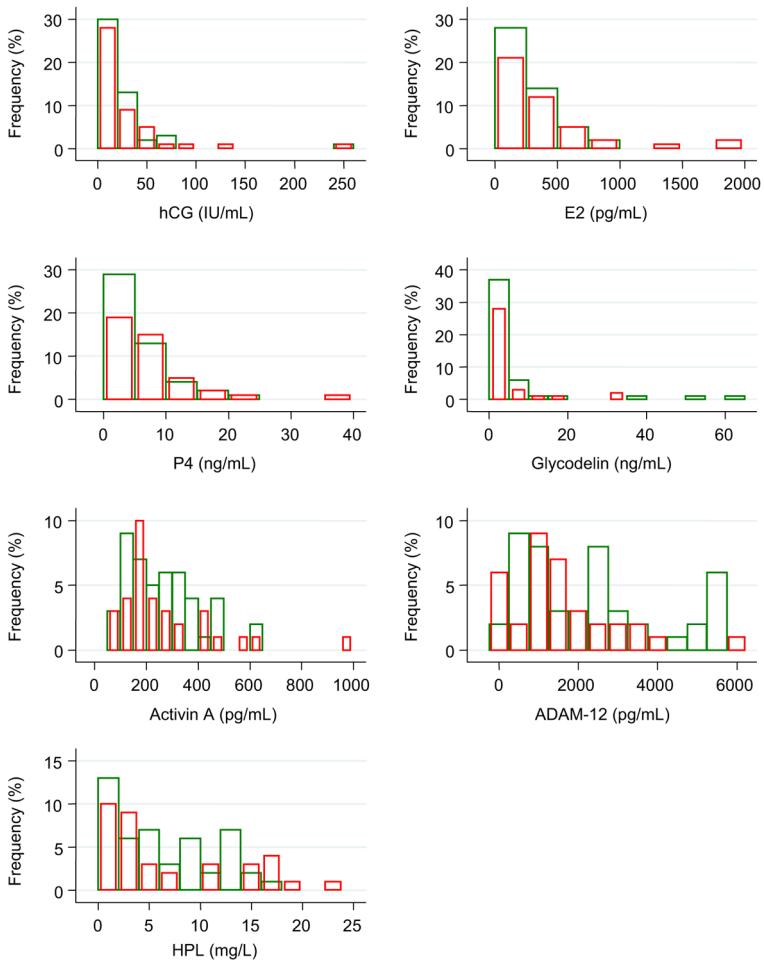

Fig. 1 –.

Biomarker frequency distributions for tissue expulsion with misoprostol. Green = success; red = failure; hCG = human chorionic gonadotropin; E2 = estradiol; P4 = progesterone; HPL = human placental lactogen.

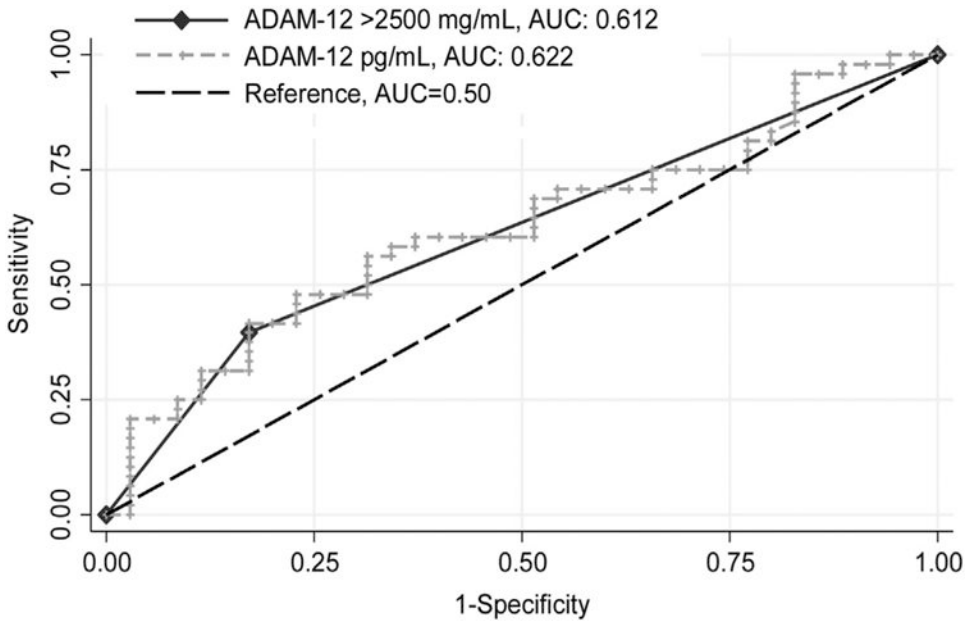

Fig. 2 –.

Receiver-Operator Characteristic (ROC) curves for ADAM 12, shown both as a dichotomous variable [≥2500 pg/mL (solid)], and a continuous variable (dash) for comparison. The reference line is a long dash.

Table 1 –

Demographic and clinical characteristics of the study sample.

	Total sample (*n* = 95)	**Success (*n* = 49)**	**Failure (*n* = 46)**	***P*-Value[a]**
Age (years), mean ± SD	28.4 ± 6.9	28.5 ± 6.6	28.3 ± 7.3	0.902
Race, *n* (%)				0.505
White	48 (50.5)	27 (55.1)	21 (45.7)	
Black	24 (25.3)	10 (20.4)	14 (30.4)	
Other	23 (24.2)	12 (24.5)	11 (23.9)	
Hispanic ethnicity, *n* (%)	33 (34.7)	11 (22.5)	22 (47.8)	0.009
Body Mass Index (BMI), *n* (%)				0.399
Normal	44 (46.3)	26 (53.1)	18 (39.1)	
Overweight	30 (31.6)	13 (26.5)	17 (37.0)	
Obese	21 (22.1)	10 (20.4)	11 (23.9)	
Number of prior miscarriage, *n* (%)				0.692
0	71 (74.7)	37 (75.5)	34 (73.9)	
1	16 (16.8)	7 (14.3)	9 (19.6)	
2	8 (8.4)	5 (10.2)	3 (6.5)	
Parity, *n* (%)				0.010
0	37 (38.9)	24 (49.0)	13 (28.3)	
1	32 (33.7)	18 (36.7)	14 (30.4)	
2+	26 (27.4)	7 (14.3)	19 (41.3)	
Type of pregnancy, *n* (%)				0.545
Embryonic/fetal demise	59 (62.1)	29 (59.2)	30 (65.2)	
Anembryonic gestation	36 (37.9)	20 (40.8)	16 (34.8)	
Duration of bleeding (days), mean ± SD	4.8 ± 1.3	4.7 ±1.3	4.8 ± 1.3	0.626
Lower abdominal pain in last 24 h, *n* (%)	58	30 (61.2)	28 (60.9)	0.972
Vaginal bleeding in last 24 h, *n* (%)	55	32 (65.3)	23 (50.0)	0.131
Baseline gestational sac mean diameter,[b] mean ± SD	24.7 ± 10.7	23.1 ± 8.8	26.4 ± 12.3	0.613

[a]Comparison of success versus failure using Mann-Whitney *U*-, Chi-square or Fisher's exact tests.

[b]*n* = 58.

Table 2 –

Biomarker levels categorized by complete or incomplete miscarriage with one misoprostol dose.

	Total sample (*n* = 95)	Success (*n* = 49)	Failure (*n* = 46)	*P*-Value[a]	AUC
Trophoblastic proteins					
Activin A (pg/mL),[b] *n* (%)				0.351	0.53
<240	44 (55.0)	24 (51.1)	20 (60.6)		
240 to <540	31 (38.8)	21 (44.5)	10 (30.3)		
≥540	5 (6.3)	2 (4.3)	3 (9.1)		
Glycodelin (ng/mL),[b] *n* (%)				0.336	0.55
<3	59 (71.1)	32 (66.7)	27 (77.1)		
≥3	24 (28.9)	16 (33.3)	8 (22.9)		
ADAM-12 (pg/mL), *n* (%)				0.032	0.61
<2500	58 (69.9)	29 (60.4)	29 (82.9)		
≥2500	25 (30.1)	19 (39.6)	6 (17.1)		
HPL (mg/L), *n* (%)				0.059	0.51
<5	42 (50.6)	22 (46.8)	20 (55.6)		
5 to <15	31 (37.4)	22 (46.8)	9 (25.0)		
≥15	10 (12.1)	3 (6.4)	7 (19.4)		
Hormones					
E2 (pg/mL), *n* (%)				0.294	0.54
<500	75 (81.5)	42 (85.7)	33 (76.7)		
≥500	17 (18.5)	7 (14.3)	10 (23.3)		
P4 (ng/mL), *n* (%)				0.424	0.53
<10	76 (82.6)	42 (85.7)	34 (79.1)		
≥10	16 (17.4)	7 (14.3)	9 (20.9)		
hCG (baseline value; mIU/mL), *n* (%)				0.061	0.58
<4000	17 (17.9)	5 (10.2)	12 (26.1)		
≥4000	78 (82.1)	44 (89.8)	34 (73.9)		

AUC: area under the ROC curve; HPL: human placental lactogen; E2: estradiol; P4: progesterone.

[a]Comparison of success vs. failure using Fisher's exact test.

[b] Activin A: $n = 80$; glycodelin: $n = 83$.

Table 3 –

Final logistic predictive model for successful uterine evacuation after one dose of misoprostol.

	Odds ratio	95% CI	P-Value
Hispanic	0.16	0.04–0.56	0.004
Parity			
0	(ref)		
1	0.86	0.25–2.97	0.814
2+	0.22	0.06–0.88	0.032
hCG (mIU/mL)			
<4000	(ref)		
≥4000	4.80	1.17–19.67	0.029
ADAM-12 (pg/mL)			
<2500	(ref)		
≥2500	5.19	1.30–20.64	0.019
Obese			
No	(ref)		
Yes	2.80	0.69–11.41	0.151

CI: confidence interval.