

# Factors affecting retention and compliance in a longitudinal study of connected, low income, urban, primiparous mothers

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## Abstract

**Background/Aims:** Longitudinal obstetrics studies are vital to our understanding of the physiological and social changes that occur during pregnancy and the early postpartum period in the mother and baby. However, the passage of time and other factors including loss of interest in study participation and changes in subject availability may affect retention rates. Here variables collected in a longitudinal obstetrics study were analysed to identify factors affecting retention and compliance within a low-income, primiparous population.

**Methods:** Primiparous women were recruited for a prospective cohort longitudinal study. Two study arms were used. The first collected survey data on mood, sleep, and night time eating and actigraphic data for seven days. The second was identical to the first, but had an additional 24-hour sampling of saliva. Data were collected during three time

**Points:** weeks 22 and 32 of gestation and one week postpartum. Pick up and drop off of study materials at the research site were required for each time point. Subjects were given the option to complete surveys online, by phone, or by mail. In addition, breast fullness surveys were administered by phone, email, or text each day for the first five days postpartum.

**Results:** Ninety-two women were recruited, of which 45% were retained and compliant for the entire study. Demographics of the enrolled and retained cohorts were similar and presented as being primarily low-income, Black or African American (58.7% enrolled, 63.4% retained) women, with a mean age of 23 years. The majority of subjects (88%) had daily internet access, completed surveys on-line (81.4%), and preferred to receive text messages (93.5%) for study reminders as compared to other methods of communication. Longitudinal time ( $P<0.001$ ), increased number of reminders ( $P<0.001$ ), and increased length of time to complete surveys ( $P<0.001$ ) had a significant negative effect on study retention, whereas enrolment in the saliva study arm with greater sampling and more communication ( $P<0.001$ ) and earning a higher percentage of available compensation based on compliance with study protocol ( $P<0.001$ ) had a significant positive effect on study retention.

**Conclusions:** The high rate of daily internet access and preference for text messaging for primary means of communication with research staff suggests a high rate of smart-device technology among relatively young, urban-dwelling, low income women. Designing studies that can be completed via the web and using text message reminders may be a preferable and a practical means of conducting longitudinal obstetrics studies.

## Background/Aims

Pregnancy and the early postpartum period are times of enormous biological and social transformation for both mother and baby, and longitudinal obstetrics studies are vital for increasing the understanding of these complex changes. By using the same cohort at multiple time points, longitudinal studies allow each subject to serve as an individual control to minimize variation and better focus on the effect of time [1]. It is the passage of time which poses the most significant challenge to conducting longitudinal studies [1,2], as each subject must be willing and able to participate for an extended period. Individuals approached for participation in a longitudinal study may decline based on concerns related to the time commitment required for study completion [2]. Subjects who had previously agreed to participate in research may lose

interest between their initial enrolment and the onset or continuation of study protocol [3]. Transportation difficulties have also been cited as a major barrier to successful completion of longitudinal studies [4-6]. Life changes such as changes in employment or student status, residence, or health of the mother or infant all pose challenges to

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completion of longitudinal studies [5,6].

Successful completion of longitudinal studies may also be affected by communication method used between the research team and subjects[7], as well as the quality of communication between subjects and study staff [8]. Although the method of communication used to contact research subjects affects participation rates, it has been

found not to impact study outcomes [7]. Among low-income and ethnic minority populations, changes in the feasibility of consistent communication are common due to changes in contact information [6,9]. This is a difficult challenge for longitudinal studies, which require continuing communication with subjects over time.

Examination of successful strategies for retention of pregnant women in clinical research studies to determine the most effective and cost-efficient approaches will allow for better design and implementation of research protocols. Here we analyze variables collected in a longitudinal obstetrics clinical research pilot study to identify factors affecting retention and compliance within a low-income, primiparous population of women.

## Methods

### Subjects, setting, and study design

A prospective cohort longitudinal study following pregnant women from week 22 of gestation to one week postpartum was conducted from August 2014 to October 2015. The study was approved by Institutional Review Boards (IRB) at Purdue University, Indiana University, and Eskenazi Health (#1405014855). The overall aim of the study was to determine if circadian disruption during gestation is related to lactogenesis. Subjects were recruited from Eskenazi Hospital, a health system serving an urban, low-income population in the Indianapolis area. Inclusion criteria for the study were primiparous women, aged 18-40 years, gestational age ≤22 weeks, fluent in English or Spanish, and planning on delivering at Eskenazi Hospital.

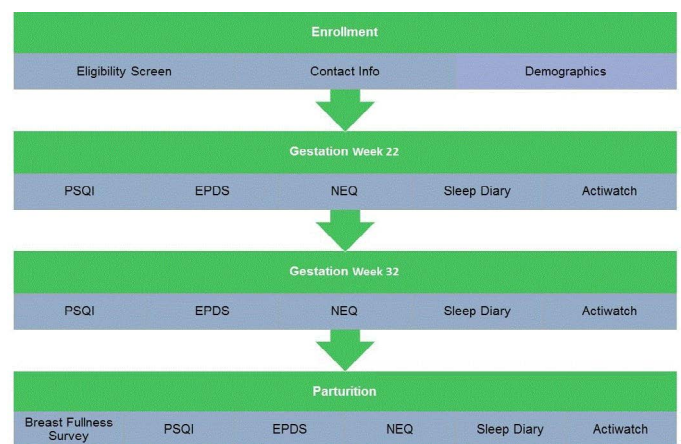
### Procedures of study protocol

Each woman who attends Eskenazi Health clinics for prenatal care is provided with an opportunity to meet with representatives from the research staff to discuss potential in clinical research studies. In order to eliminate language barriers to study participation [4], all study materials were available in both English and Spanish. Bilingual research assistants were responsible for communication with Spanish-speaking subjects. Other languages were not included due to lack of research personnel fluent in those languages.

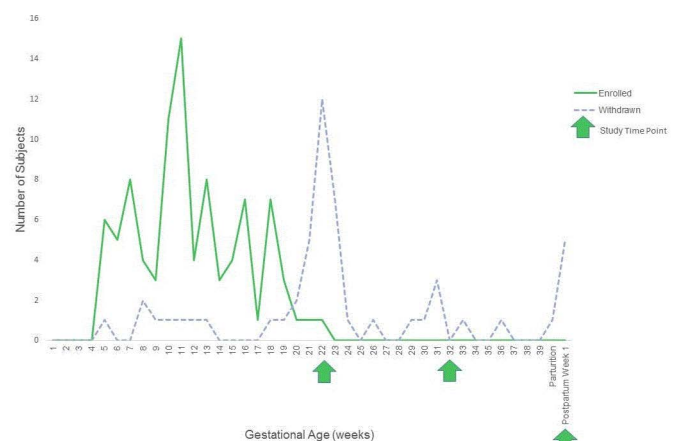
Women were enrolled in one of two arms (“saliva” or “general” arm) of the study after informed consent. The saliva arm of the study had an additional component: collecting saliva samples over a 24-hour period. This was accompanied by an increased incentive compensation for subjects in this arm. Women were offered the option of saliva or general arm enrollment until enrollment goals for the saliva arm were met. Thereafter women were only offered enrollment in the general arm. Upon obtaining informed consent, subjects were asked to complete an intake survey that collected data on demographics, internet access, contact information and preferred primary communication method (text messaging, voice phone calls, or email) to arrange pick-up and drop-off of study materials that included a sleep diary and a wrist actigraph (Actiwatch, Philips Respironics, Andover, MA) for measurement of sleep and activity.

Each woman was sent a personalized welcome message from the

research team via email or text message following enrollment. Data collection began at 22 weeks gestation and continued until one week postpartum (Figure 1). All subjects were asked to wear an Actiwatch for seven consecutive days during weeks 22 and 32 of gestation and again one week postpartum, as well as to keep a sleep diary during that time (Figure 1). At enrollment all subjects were also asked their preferred method to complete validated surveys of maternal mood (Edinburgh Postnatal Depression Scale, EPDS [10]), sleep quality (Pittsburgh Sleep Quality Index, PSQI [11]), and night time eating (Night-time Eating Questionnaire, NEQ[12]) during weeks 22 and 32 of gestation and post-partum week one. Choices given to complete studies were on-line via survey administered with Qualtrics software (Provo, Utah), by phone, or via mail-in hard copy. Subjects in the saliva arm were given a sample collection kit and asked to collect saliva every four hours over a 24-hour period, record collection time, and store samples in a freezer (in subjects’ home) during weeks 22 and 32 of gestation and in the hospital beginning 24 hours after the birth of their infants.



**Figure 1.** Study Outline. Study outline includes enrollment and the three subsequent time points, with a list of the study activities required to be completed at that time point. Saliva sample collection (not included) for women in the saliva arm of the study also occurred at each of the three time points following enrollment. Validated survey instruments included: PSQI: Pittsburgh Sleep Quality Index; EPDS: Edinburgh Postnatal Depression Scale; NEQ: Night Eating Questionnaire.



**Figure 2.** Recruitment and Retention. (a) Gestational Week at Enrollment and Withdraw. Enrollment (black line) and withdrawal (gray line) of subjects by gestational week. Arrows indicate the week of each study time point. (b) Subject Retention by Arm Enrollment. Kaplan-Meier curve of gestational age at withdraw for both the saliva (light gray line) and general (dark gray line) arms of the study. Two sample proportion test was used to compare the retention rate between two groups, and analysis found retention was significantly higher in the saliva arm as compared to the general arm of study participation ( $P < 0.001$ ).

Each subject was visited in the hospital by a research assistant within 36 hours of the birth of her baby. During this visit the research assistant administered post-partum day one breast fullness survey [13] and asked the subject’s preferred method of completing the survey each subsequent day through post-partum day 5. Choices given to complete the survey were via text message, voice phone call, or email. To insure collection of these data, multiple attempts were made by the preferred communication method as well as using other methods of communication when the preferred method was unsuccessful.

For the first two study time points, subjects were contacted by a member of the research team approximately one week before scheduled data collection began to arrange for a time to pick up study materials. Each subject was given an instructional handout with information on how to wear the Actiwatch and contact information for the research team in case she had questions or the watch malfunctioned. Subjects in the saliva group were given a collection kit and instructions on how to collect saliva samples. Arrangements to drop off the Actiwatch were made at the time of pick up or via phone call or text to the subject by a member of the research team near the end of the data collection period. Efforts were made to meet subjects at their scheduled prenatal appointments to make study participation more convenient; however, subjects were informed during enrolment that additional visits to the study site may be required if their scheduled appointments did not coincide with study time points. During the third time point a member of the research team met each subject in her hospital room to deliver the study materials; return of materials to the study site was arranged with the research assistant at that time.

**Compliance and retention**

Adherence to study protocol, or compliance, is important for accurate evaluation of investigational outcomes [14]. Compliance was defined as completing all required study elements. At the time of study material drop-off, subjects were given full compensation if they were compliant with all study protocols.

Partial compensation was given to subjects who completed at least half of the study requirements. In order to determine the level of compliance, saliva samples were counted, if applicable; surveys were checked for completion; and Actiwatch data was examined before compensation was given. Subjects were given the choice of gift cards from three local retailers.

**Statistical analysis**

We analyzed the effect of the method of survey administration (online, in person, or by phone), communication preferences (method), study arm enrollment, number of reminders sent to subjects, attendance of prenatal care appointments, and compensation on rates of retention and compliance using SPSS 23 (IBM, Armonk, NY) to conduct the following statistical tests: t-test, one and two sample proportion z-tests, Kaplan-Meier survival analysis, linear regression, and chi-squared tests. Analysis method used is specified within the text.

**Results**

**Subject demographics and longitudinal retention rates**

Ninety-two primiparous women were recruited for the study. The ages of subjects who enrolled in the study ranged from 18-33 years, median age 22.5 years. Gestational age at enrollment ranged from 5-22 weeks, mean gestational age of 11.73 weeks (Figure 2a). The recruited

population was fairly homogenous, 58.7% self-reported their race as “Black or African American,” 63% with High School diploma or GED being the highest educational level attained, 46.7% earning less than \$10,000 per year, and 65.2% working for wages (Table 1). Employment status of homemaker and lower educational attainment may be factors affecting retention; however, it is difficult to draw conclusions based on the low number of women in these demographic groups in our study. There was no effect of age, race or yearly household income on retention. Analysis of withdraw rates among women who enrolled between 5-13 and 14-22 weeks’ gestation showed that there was no effect of gestational age at recruitment, with 53% of subjects withdrawing in both populations.

Fifty-five percent (n=49) of the initially enrolled population withdrew from the study, with twenty-five percent of the enrolled population (n=23) withdrawn from the study by members of the research staff due to failure to comply with study protocol (Table 2). Examples of noncompliance included failure to wear the Actiwatch for the minimum five of the seven requested days, failure to complete the surveys in the requested timeframe, and failure to communicate with members of the research staff to arrange pick up of study materials. Changes in health status or other life events prevented 21 women from completing the study (22.8% of enrolled population, 41% of withdraws). The remaining 13.7% of women who did not complete the

**Table 1.** Demographics of Recruited, Retained, and Withdrawn Subjects.

	Recruited N=92	Retained N=41	Withdraw N=51	Difference Between Withdraw and Retained
	No. (%)	No. (%)	No. (%)	P-value <sup>1</sup>
<b>Race</b>				
Asian	1 (1.1)	1 (2.4)	0 (0.0)	0.2660
Black or AfricanAmerican	54(58.7)	26 (63.4)	28 (54.9)	0.4105
White	23(25.0)	8 (19.5)	15 (29.4)	0.2756
More than one race	12 (13.0)	4 (9.8)	8 (15.7)	0.4040
Unknown/notreported	2 (2.2)	2 (4.9)	0 (0.0)	0.1100
<b>Educational Attainment</b>				
Less than 7th grade	2 (2.2)	1 (2.3)	1(2.0)	0.9212
Junior High (9th grade)	1 (1.1)	1 (2.3)	0 (0.0)	0.2763
Partial High School (10th or 11th grade)	8 (8.7)	0 (0.0)	8 (15.7)	0.0079
High SchoolGraduate or GED	58 (63.0)	27 (62.8)	31 (60.8)	0.8845
Associate degree (2 years)	8 (8.7)	5 (11.6)	3 (5.9)	0.3283
Bachelor's degree (4 years)	8 (8.7)	6 (14.0)	2 (3.9)	0.0826
Graduate Degree	8 (8.7)	6 (14.0)	2 (3.9)	0.0826
<b>Yearly Household Income</b>				
Less than \$10,000	43(46.7)	19 (44.2)	24 (47.1)	0.7814
\$10,000-\$24,999	21 (22.8)	9 (20.9)	12 (23.5)	0.7660
\$25,000-\$49,999	19 (20.7)	9 (20.9)	10 (19.6)	0.8773
\$50,000-\$74,999	3 (3.3)	2 (4.7)	1 (2.0)	0.4648
Greater than \$74,999	4 (4.3)	2 (4.7)	2 (3.9)	0.8502
<b>Employment</b>				
Homemaker	4 (4.3)	4 (9.3)	0 (0.0)	0.0261
Out of work, but NOT looking for work	5 (5.4)	3 (7.0)	2 (3.9)	0.5088
Out of work and looking for work	21 (22.8)	8 (18.6)	13 (25.5)	0.4303
Self-employed	1 (1.1)	1 (2.3)	0 (0.0)	0.2763
Working for wages	60. (65.2)	27 (62.8)	33(64.7)	0.8505

Two sample proportion z-test was used to analyze differences between recruited subjects and retained subjects.

**Table 2.** Reasons for Study Withdraw.

Reason for Withdraw	No. (% of Enrolled)		Percent of Withdraws
Loss of pregnancy or Poor prognosis of infant	7	(7.6)	13.7%
Changed mind about participating in study	7	(7.6)	13.7%
Change of hospital	14	(15.2)	27.5%
Not compliant with study protocol	23	(25.0)	45.1%

study were eligible for study participation, but changed their minds about participating after enrollment.

Analysis of withdrawal rates across time showed that 24% of withdrawals occurred before the study began, and 47% withdrew by declining to proceed with study procedures at the first time point, i.e. between weeks 21 and 23 of gestation (Figure 2a). Between the first and third time points 16% of withdrawals occurred, and approximately 14% of withdrawals occurred coincident with the post-partum time point. Thus the majority of withdrawals occurred before completion of the first time point ( $P < 0.001$ , chi-squared test). In general, within a study time point, study withdrawals occurred during the week before the data collection period. The most common reason cited by subjects for withdrawal was an inability of subjects to arrange pick-up of study material. Withdrawals following the study time-point were due to failure of subjects to comply with study protocol (Table 2).

There was a significant effect of study arm on withdraw rate ( $P < 0.001$ , chi-squared test; Figure 2b, Kaplan-Meier). The arm requiring saliva sampling in addition to survey completion and collection of actigraph data accounted for 56% of the subjects who successfully completed the study, despite accounting for only 37% of the enrolled population. The association of study arm with retention rates led us to analyze factors which differed between the groups: communication and compensation.

**Communication in relation to recruitment, retention and compliance**

Subjects were polled during intake as to whether they had internet access, and if they did, whether their access was daily. The majority of subjects (88%) had daily internet access (Figure 3). During intake, subjects were also asked their preferred method of communication (phone, text, or email) for study reminders and arranging pick-up and drop-off of study materials. The majority of recruited (93.5%) and retained (93.0%) subjects requested to receive text messages from the research staff. Email reminders were requested by 9.9% of the women. These data demonstrate that this population is “connected,” with the majority having daily access to the internet and preference for text messaging versus other forms of communication (Figure 3). The research team received more phone calls from women enrolled in the first, more involved study arm (mean 2.5 calls vs. mean 0.32 calls per subject;  $P = 0.001$ , t-test) during sampling periods, with no significant difference in text messaging rates between the two groups ( $P > 0.05$ , t-test).

**Survey method in relation to recruitment, retention and compliance**

For each time point, subjects were asked how they preferred to complete study surveys and were given the option to complete surveys on-line, via phone call, or by mail. The option to complete study surveys online was most popular; 80.4% of enrolled women indicated a preference for completing surveys online. The remaining women completed surveys by phone or in person. Although 6.5% signed up to receive and return surveys via mail, no subject completed surveys by

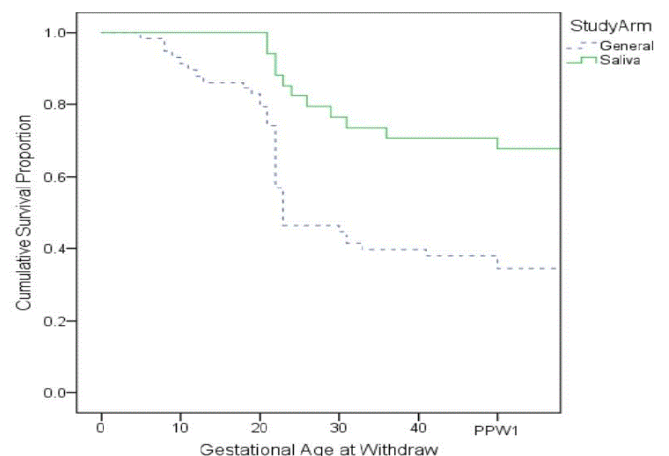
this method. Alternatively, an additional 6.5% of subjects completed hard-copies of surveys during site visits for drop-off of study materials. Although the majority of the retained cohort (83%) completed surveys online, there was no significant correlation between survey method and successful study completion ( $P > 0.05$ , chi-squared test).

There was also no significant difference in preferred survey method between the two study arms ( $P > 0.05$ , chi-squared test). The mean number of reminders sent to subjects who completed surveys online was two (range 0 to 5), with more reminders sent to subjects who took more days to complete surveys ( $P < 0.001$ , linear regression). Subjects who needed more than two reminders ( $P < 0.01$ , chi-squared test), and subjects who took more than five days to complete the online surveys ( $P < 0.01$ , chi-squared test) were more likely to withdraw from the study. Of those who completed all online surveys within five days, 93.33% successfully completed the study. Of the 15 women who chose online survey administration but failed to complete surveys online, half were retained when given the alternative of completing the surveys by phone or in person upon study material return. Subjects who completed the online surveys more quickly did so with less involvement of the research staff, and were more likely to successfully complete the study protocol.

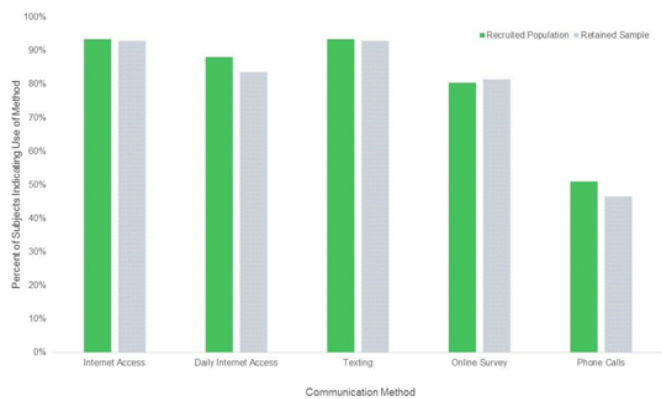
In addition, online administration was the most efficient method for subjects to complete surveys. Median time for survey completion via online administration was eleven minutes (range 5 minutes 1 second to 9 days, 19 hours, 32 minutes, 4 seconds) for all three surveys. Average time for completing three surveys via voice phone calls was estimated to be 30 minutes per time point. No surveys were returned by mail; however hard copies filled out in person at the hospital were estimated to take 15 minutes to complete all surveys per time point.

**Compensation in relation to compliance and retention**

Average compensation levels decreased over the course of the study, with the first week postpartum having the lowest proportion of subjects receiving full compensation (82%;  $P = 0.0473$ , t-test; Figure 4). Women who received 100% of the compensation for which they were eligible were more likely to remain in the study ( $P < 0.001$ , sign test). There was no significant difference in the percentage of compensation received between the two study arms ( $P > 0.05$ , one sample proportion z-test). Women in the saliva arm were more likely to complete the study than those in the general arm. Subjects in the saliva arm were eligible to



**Figure 3.** Preferred Communication Method. Subject communication preferences as indicated via the intake survey completed during enrollment. For each method of communication, the green bar represents the percentage of recruited population and the textured bar represents the percentage of the retained population.



**Figure 4.** Compensation Decrease over Time. On each of the time point, the left green bar is number of subject that get full compensation, the middle one is number of subjects get partial compensation and the left bar is the number of subjects didn't get compensation. Two sample proportion test found the number of subjects, who received full compensation is significantly higher than the number of subject who received partial or no compensation ( $P < 0.001$ ). Analysis of time points on proportion of subjects receiving full compensation, found a significant decrease from gestation week 22 to gestational week 32, and from gestational week 32 to postpartum week 1 ( $P < 0.001$ ).

receive a higher maximum compensation amount at each time point.

## Discussion

Our study was very involved, requiring at least five site visits for pickup and drop-off of study materials. Obstetric studies with similar designs have reported a range of 41% to 62% retention, with an average retention rate of 50% [7,15-18]. Clinical studies focused on low income populations historically have lower compliance and retention rates compared to studies of higher socio-economic populations [19]. A study that followed a low-income cohort for one-year beginning in the immediate postpartum period retained a similar percent of subjects (44%, [16]). Our retention rate was lower than the 59% reported for a longitudinal study of low income obstetric subjects with six time points; however, all of the three prenatal and three postnatal time points in that study were conducted in the subjects' homes [15]. Removing the transportation barrier may have helped increase retention in their study relative to ours, as a pilot study of well- educated, white primiparous women which required four site visits achieved a similar retention rate (43%) to ours (45%) [18].

We had a loss of subjects over time, which is consistent with the longitudinal design of the study. The greatest withdrawal rate coincided directly with the first study time point, with the primary reasons for subject withdraw being noncompliance with study protocol and change of hospitals (Table 2). In addition to the high withdrawal rate associated with the first study time point, there was also a drop in participation during the first week post-partum. We believe this drop in participation was related to the overwhelming nature of this period in a new mother's life [8]. Despite loss of half of subjects over the course of the study, we found being flexible with the return of study materials and meeting women at their regular care appointments allowed us to achieve 100% return rate of study materials that were distributed, even from women who declined continued participation.

In addition to the effect of time on subject retention, retention for subjects enrolled in the more involved protocol was 67% as opposed to 31% for the less involved protocol. Compensation was higher in this arm, but there was also more contact time with the research team, and more calls placed to members of the research team by subjects enrolled in this arm. Sharps et al. had a similar study design with two separate

study arms, one of which required more participation from subjects. Consistent with our findings, they found 5% higher retention in the more involved group [15]. The greater amount of communication required for the saliva arm likely enhanced retention rates as it allowed for further development of interpersonal relationships between subjects and research staff. A potential limitation of our conclusion is that the more intensive arm of the study was filled first, and although women enrolled in the general arm after the saliva arm was full were unaware of the existence of a separate arm receiving higher compensation, it is possible that in addition to differences in communication inherently required by the additional study protocol women in the saliva arm may have received more attention and been more closely followed by members of the research team.

Despite the low income status of the overwhelming majority of subjects in our study, these women were very "connected" (93.5% preferred text messaging as primary method of communication, 88% had daily internet access). Online surveys were used by 81% of women who completed the study. Given a preference for texting and daily internet access, we assume that women were using smartphones/smart devices to complete study procedures. Others have reported that low-income, African American women are commonly dependent on mobile technology for internet access as well as telephone communication [9,20]. Therefore, one factor impacting retention and compliance for longitudinal studies in low-income demographic populations is the continuation of cell phone service, which facilitates contact with study subjects over time [9,21].

The majority (93%) of our population indicated a preference for text messaging to communicate with research staff. Text messaging has been established as an effective tool for clinical and research purposes in low-income, African American populations [20,22-24], as well as for obstetrics research projects, with the Feeding Your Baby Project in Scotland also reporting a 93% response rate using text messaging as the exclusive means of data collection [23]. While text messaging was a convenient and effective method for communicating with subjects in our study, we found no effect of communication method or text message use on retention rates.

We used individualized messages instead of automated messages for all communication with study subjects. This more personal approach has been shown to be preferred by subjects and important for cohort maintenance in previous clinical research studies [4,8,19]. The development of personal relationships between subjects and study staff has been described as an integral factor in recruitment and retention of subjects in longitudinal obstetrics studies [19]. In addition to contacting the subjects, the research team was often contacted by the study subjects, which indicated that they felt comfortable with the research staff and liked developing interpersonal relationships with them. We believe the increased interaction between subjects and the research team to be particularly influential on subject retention in the more involved arm of the study.

Using Qualtrics (Qualtrics LLC, Provo, UT) to administer surveys was most efficient for research staff. Use of online surveys was not correlated with a higher retention rate, but it remained a popular option with both the enrolled and retained population (83% of the retained cohort and 78% of the withdrawn cohort). Qualtrics records time to complete surveys based on how long the program running the survey remains active [25]. Subjects who delayed survey completion received additional reminders from the study staff; both factors were correlated with a decreased likelihood of study completion. Requesting

completion of online surveys by potential subjects as a screening tool for clinical research studies may be a useful method of indicating which individuals are more likely to be retained through study completion.

Previous findings related to the impact of compensation on clinical research subject retention are mixed [26,27]. In our study, there was no difference in the percentage of compensation received between the two arms; however, subjects who received 100% of the compensation for which they were eligible were more likely to successfully complete the study. The greatest rate of partial compensation was at the postpartum time point. Since partial compensation was given for partial compliance, the higher rate of partial compensation likely reflects additional burden of study participation for women in the immediate postpartum period.

## Conclusion

This highly connected, low-income, primiparous obstetric population preferred text messages for communications with the research staff. Additionally, the majority of subjects preferred to complete study surveys online. Using personal text messaging and email to communicate enabled the development of a relationship between subjects and the research team, which facilitated cohort retention. The largest loss of subjects occurred near the first study time point, when subjects realized the extent of activities required for study involvement. A means of identifying this early withdraw group may help to estimate recruiting goals. Screening for subject completion of intake survey data within five days with two or fewer reminders from research staff may indicate subjects who are more likely to successfully complete study protocol. The use of personal communication, adequate participant compensation, and utilizing mobile and online platforms for study survey completion are all ways to enhance longitudinal obstetric cohort retention.

## Author contributions

AB, collected and analyzed data and drafted manuscript; HS, collected and analyzed data and edited manuscript; SC, collected and analyzed data, JC, collected and analyzed data and edited manuscript; SD, collected and analyzed data; LZ, designed study, collected and analyzed data and drafted manuscript; DH, designed study, collected and analyzed data and edited manuscript; TC, designed study, collected and analyzed data and drafted manuscript; AA, designed study, collected and analyzed data and drafted manuscript.

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