OBSTETRICS

Obesity and cell-free DNA "no calls": is there an optimal gestational age at time of sampling?

Mary C. Livergood, MD; Kay A. LeChien, MS, CGC; Amanda S. Trudell, DO, MSCI

BACKGROUND: Cell-free DNA screen failures or "no calls" occur in 1-12% of samples and are frustrating for both clinician and patient. The rate of "no calls" has been shown to have an inverse relationship with gestational age. Recent studies have shown an increased risk for "no calls" among obese women.

OBJECTIVE: We sought to determine the optimal gestational age for cell-free DNA among obese women.

STUDY DESIGN: We performed a retrospective cohort study of women who underwent cell-free DNA at a single tertiary care center from 2011 through 2016. Adjusted odds ratios with 95% confidence intervals for a "no call" were determined for each weight class and compared to normal-weight women. The predicted probability of a "no call" with 95% confidence intervals were determined for each week of gestation for normal-weight and obese women and compared.

RESULTS: Among 2385 patients meeting inclusion criteria, 105 (4.4%) had a "no call". Compared to normal-weight women, the adjusted odds ratio of a "no call" increased with increasing weight class from overweight to obesity class III (respectively: adjusted odds ratio, 2.31; 95% confidence interval, 1.21—4.42 to adjusted odds ratio, 8.55; 95% confidence interval, 4.16—17.56). A cut point at 21 weeks was identified for obesity class II/III women at which there is no longer a significant difference in the probability of a "no call" for obese women compared to normal weight women. From 8-16 weeks, there is a 4.5% reduction in the probability of a "no call" for obesity class II/III women (respectively: 14.9%; 95% confidence interval, 8.95—20.78 and 10.4%; 95% confidence interval, 7.20—13.61; $P_{trend} < .01$).

CONCLUSION: The cut point of 21 weeks for optimal sampling of cell-free DNA limits reproductive choices. However, a progressive fall in the probability of a "no call" with advancing gestational age suggests that delaying cell-free DNA for obese women is a reasonable strategy to reduce the probability of a "no call".

Key words: aneuploidy screening, cell-free DNA screening, "no call", noninvasive prenatal screening, noninvasive prenatal testing, obesity

Introduction

Despite controversy as to the utility of cell-free DNA (cfDNA) as a universal first-trimester screening approach, cfDNA has been popularized and is being utilized as aneuploidy screening for both high- and low-risk women throughout the United States. The majority of women who undergo cfDNA will have a clinically useful result of positive or negative; however, 1-12% of women and their clinicians will be frustrated by a cfDNA report without a result or a "no call."[1] It is well established that the risk of a "no call" falls with advancing gestational age in association with an increase in the circulating fetal fraction of cfDNA in maternal serum.[2-4] Additionally, maternal weight has been shown to be inversely related to the fetal fraction of circulating cfDNA.[2,3,5] A low fetal fraction, usually <4%, can result in a "no call".

Recently, the significance of a "no call" has come to light as high rates of aneuploidy have been reported among pregnancies with a "no call" with odds ratios (ORs) for aneuploidy ranging from 2.5-9.2, depending on whether the fetal fraction is taken into account.[6] With increasing use of cfDNA for aneuploidy screening and increasing rates of maternal obesity, the "no call" has become a common problem proving to hold increasing clinical significance. With the inverse relationship between gestational age and "no call" risk and the direct relationship between maternal weight and "no call" risk, the question remains as to whether delaying cfDNA sampling in obese women might reduce the risk of a "no call". Therefore, our objective was to determine the optimal gestational age for cfDNA in obese women to minimize the risk of a "no call".

Materials and Methods

We conducted a retrospective cohort study of prospectively collected data from the perinatal genetics database at Mercy Hospital St Louis. Institutional review board approval was obtained prior to beginning the study. Mercy Hospital St Louis serves as a large community tertiary referral center in St Louis, MO. Our obstetric service performs approximately 8500 deliveries annually with 34,895 births and 35,937 neonates born during the 5-year study period.

The perinatal database is maintained prospectively by 3 board-certified genetic counselors. A total of 5602 patients underwent complete genetic counseling at Mercy Hospital St Louis during the study period and were offered traditional serum screening, cfDNA screening, and diagnostic testing. In all, 2390 patients chose cfDNA and, while it is unknown whether insurance or rebates paid for their testing, all patients were accepting of their out-of-pocket costs. Women who received a "no call" result were offered diagnostic testing in response to the result, but given there is no current standard as to the best management of a "no call", the options of traditional serum screening as well as a cfDNA redraw were also discussed and the patients were counseled extensively by the

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genetic counselors as to the risks and benefits of each approach. Maternal demographic characteristics, date of cfDNA, gestational age at time of cfDNA, modality of cfDNA utilized, and result were collected and entered into the database. Gestational age was based on the best obstetrical estimate.[7] The study included all women who underwent cfDNA through our perinatal genetics department from Nov. 30, 2011, through March 15, 2016. Five women missing either height and/or weight data were excluded from the study.

The patient's height and weight at the time of cfDNA was collected from the electronic medical record. Weight was either obtained from the patient's primary obstetrician's office record if she had an appointment within 3 days of her cfDNA or self-reported. Weight and height were used to calculate body mass index (BMI) and women were classified based on the World Health Organization obesity classification system. BMI $\leq$18.5 kg/m^2 was classified as underweight, BMI 18.5-24.9 kg/m^2 as normal weight, BMI 25-29.9 kg/m^2 as overweight, BMI 30-34.9 kg/m^2 as class I obesity, BMI 35-39.9 kg/m^2 as class II obesity, and BMI $\geq$40 kg/m^2 as class III obesity.

Maternal demographic information was compared among women with a reportable result and women with a "no call". Univariate analysis was performed using χ^2 for categorical variables, Student *t* test for parametric continuous variables, and Mann-Whitney *U* test for nonparametric continuous variables.[8] Variables with multiple groups were compared using the nonparametric test for trend described by Cuzick.[9]

Logistic regression was used to determine the OR with 95% confidence intervals (CIs) for a "no call" for each BMI category. Multivariable logistic regression was used to control for maternal age. Adjusted OR (aOR) with 95% CIs for a "no call" compared to normal-weight women were then determined for each BMI category. The predicted probability of a "no call" with 95% CIs was calculated throughout gestation stratified by weight class and compared between normal-weight women and overweight/obese women.

TABLE 1
Comparison of maternal characteristics of women with reportable result on initial cell-free DNA draw and those with "no call"

Maternal characteristics	Reportable n = 2280	No call n = 105	*P*
Maternal age at delivery, y	34 (29–37)	36 (31–38)	.030
AMA	915 (40.13)	53 (50.48)	.035
GA at initial draw, wk	12.71 (11.57–17.57)	12.57 (11.57–14.71)	.43
BMI kg/m^2, mean (SD)	27.71 (6.84)	33.39 (8.44)	<.05
Multiple gestation	70 (3.07)	2 (1.90)	.50
Indication			P_{trend}
Low risk	824 (36.14)	28 (26.67)	
AMA alone	735 (32.23)	45 (42.86)	
Ultrasound finding	200 (8.72)	10 (9.52)	.66
Abnormal serum screen	452 (19.82)	20 (19.05)	
Other[a]	69 (3.02)	2 (1.90)	
Modality			P_{trend}
Panorama	1642 (72.02)	84 (80.00)	
Harmony	380 (16.67)	11 (10.48)	.09
Maternity 21	44 (1.93)	6 (5.71)	
Verifi	214 (9.39)	4 (3.81)	

Unless otherwise noted, values are n (%) or median (interquartile range).

AMA, advanced maternal age; *BMI*, body mass index; *GA*, gestational age.

[a] History of offspring effected by aneuploidy, parental balanced translocation increasing risk for aneuploidy, or multiple indications for noninvasive prenatal testing that do not include AMA.

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Results

Of the 2385 patients meeting inclusion criteria, 105 (4.4%) had a "no call". In all, 55.7% of women in the study were overweight or obese with more than a quarter (27%) classified as class $\geq$I obesity. On average, women with a "no call" were older than women with a reportable result by 2 years (age 36 years; IQR, 31–38 vs age 34 years; IQR, 29–37; $P = .03$). Women with a "no call" had a higher BMI than women with a reportable result, on average, the BMI of the "no call" group was in the class I obesity range compared to the reportable result group, which was in the overweight range (BMI 33.39 vs 27.71 kg/m^2, $P <$ .05). Gestational age at the time of sampling, indication for testing, and modality of testing did not differ between groups (Table 1). The average gestational age at the time of initial cfDNA was between 12-13 weeks (Table 1). The most common indication category for cfDNA was low risk (35.7%, n = 852) closely followed by advanced maternal age (32.7%, n = 780). Panorama served as the cfDNA modality for the majority of the study population (72.4%, n = 1726).

The ORs and aORs for a "no call" for each weight class are presented in Table 2. When BMI was stratified by weight class, the odds of a "no call" remained significantly >1 only among obesity class II and III women, respectively (aOR, 2.91; 95% CI, 1.74–4.85 and aOR, 3.30; 95% CI, 1.97–5.52) (Table 2). Women who fell into weight classes with a BMI <35 kg/m^2 did not demonstrate increased odds of a "no call". Compared to normal-weight women, the aOR of a "no call"

TABLE 2
Odds ratios and adjusted odds ratios for "no call" cell-free DNA stratified by body mass index class

Weight class	Reportable, n (%) n = 2280	No call, n (%) n = 105	OR (95% CI)	aOR[a] (95% CI)
Underweight BMI <18.5	26 (1.14)	1 (0.95)	0.83 (0.11–6.20)	0.86 (0.12–6.39)
Normal weight BMI 18.5–24.9	948 (41.58)	17 (16.19)	0.27 (0.16–0.46)	0.27 (0.16–0.46)
Overweight BMI 25–29.9	659 (28.9)	27 (25.17)	0.85 (0.54–1.33)	0.87 (0.55–1.35)
Class I obesity BMI 30–34.5	327 (14.34)	20 (19.05)	1.41 (0.85–2.32)	1.40 (0.85–2.31)
Class II obesity BMI 35–39.9	172 (7.50)	20 (19.05)	2.88 (1.73–4.81)	2.91 (1.74–4.85)
Class III obesity BMI ≥40	84 (6.50)	20 (19.05)	3.39 (2.03–5.67)	3.30 (1.97–5.52)

aOR, adjusted odds ratio; *BMI*, body mass index; *CI*, confidence interval; *OR*, odds ratio.

[a] Adjusted for maternal age.

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increased with increasing weight class from overweight to obesity class III, respectively (aOR, 2.31; 95% CI, 1.21–4.42 to aOR, 8.55; 95% CI, 4.16–17.56; $P_{trend} < .01$) (Table 3). For overweight and class I obese women, the CI for the predicted probabilities of a "no call" overlapped as early as 8 weeks' gestation, the earliest time cfDNA was drawn, and therefore no optimal time to reduce the probability of a "no call" was able to be determined for these weight classes (Appendices 1 and 2). For obesity class II and III women there was a significant difference in the predicted probabilities of a "no call" compared to normal-weight women until 21 and 22 weeks, respectively, at which point the CI overlapped (Appendices 3 and 4). The Figure represents the "no call" probability curves throughout gestation for women with a BMI >35 kg/m^2, obesity class II and III. For women with a BMI ≥35 kg/m^2 the cut point at which the probability of a "no call" is no longer significantly different from normal-weight women is 21 weeks of gestation, as demonstrated by the overlapping 95% CIs at this gestational age (Figure). There is a decrease in the probability of a "no call" as gestational age advances for all weight classes (Figure and Appendices 1-4). For obesity class II/III women from 8-16 weeks there is a 4.5% reduction in the probability of a "no call" (8 weeks 14.9%; 95% CI, 8.95–20.78 and 16 weeks 10.4%; 95% CI, 7.20–13.61). Sensitivity analysis demonstrated no difference in the rate of "no calls" from the first half (2011 through 2013) compared to the second half (2014 through 2016) of the study period (3.98% vs 5.51%, $P = .105$).

Comment

Our study demonstrates increased odds of a "no call" among overweight and obese women compared to normal-weight women with a significant upward trend from 2 to >8-fold as BMI increases from overweight to class III obesity. For women who have a BMI >35 kg/m^2 (class II/III obesity) the gestational age at which there is no longer a difference in the probability of a "no call" compared to normal-weight women is 21 weeks.

In a 2016 study by Norton et al,[10] the authors demonstrated 23% of chromosomal abnormalities went undetected when "no calls" were excluded in the cohort vs 29% when "no calls" were included. Further, the authors demonstrated cfDNA proved to have a lower detection rate for all chromosome abnormalities compared to traditional serum screening among the low-risk population; and the authors recommended further study on how best to evaluate "no calls".[10] Our study investigated the relationship among obesity, gestational age, and "no calls" in an attempt to determine the optimal gestational age to obtain cfDNA to reduce the probability of a "no call" among obese women. In line with the

TABLE 3
Adjusted odds ratios for "no call" compared to normal-weight women stratified by body mass index class

	Underweight N = 27	Normal weight N = 965	Overweight N = 686	Class I obesity N = 347	Class II obesity N = 192	Class III obesity N = 168
"No call", n (%) N = 105	1 (0.95)	17 (16.19)	27 (25.17)	20 (19.05)	20 (19.05)	20 (19.05)
aOR[a] (95% CI)	2.28 (0.28–18.73)	Referent	2.31 (1.21–4.42)	3.66 (1.76–7.61)	7.52 (3.60–15.74)	8.55 (4.16–17.56)
P_{trend}	<.01					

aOR, adjusted odds ratio; *CI*, confidence interval.

[a] Adjusted for maternal age.

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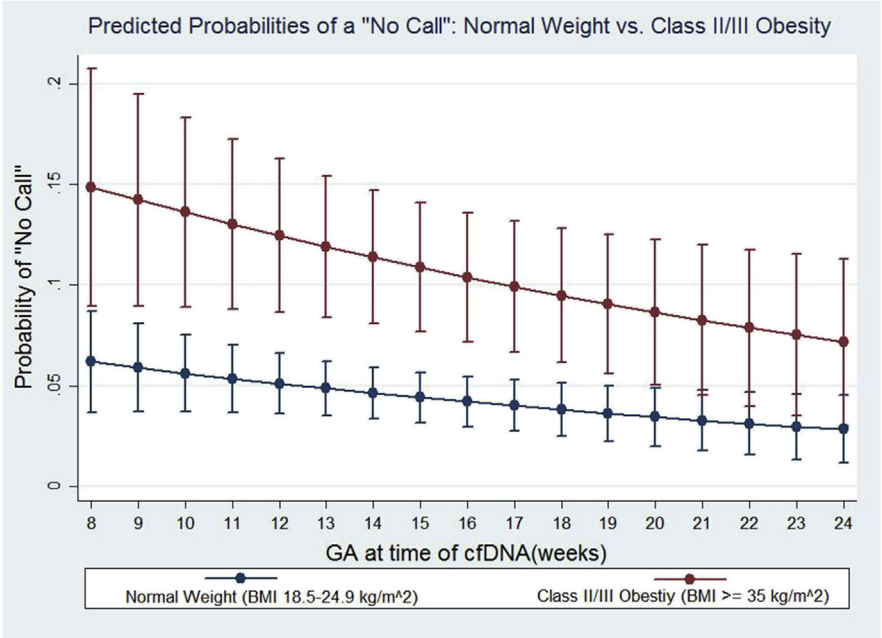
FIGURE
Predicted probabilities of a "no call": obesity class II/III



Gestational age (GA) at time of cell-free DNA (cfDNA) testing vs probability of no call for normal-weight (blue) and obesity class II and III (red) women.

BMI, body mass index.

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findings of Norton et al,[10] our study continues to elucidate the limitations of cfDNA. In context with the findings in the study of Norton et al,[10] the role of traditional serum screening and diagnostic testing, despite advances in cfDNA, is further highlighted, with traditional testing methods being particularly important for obese women.

Recent studies confirmed the association between obesity and "no calls" and the inverse relationship between gestational age and "no calls". Wang et al[2] demonstrated a negative correlation between fetal fraction and maternal weight ($P = .0003$), and reported up to 27% of the variation in the reported fetal fraction was explained by maternal weight and gestational age. Kinnings et al[3] also demonstrated an inverse correlation between fetal fraction and maternal weight (Spearman $P = -0.3934$) and described an increase in fetal fraction of 0.083% per week through 20 weeks' gestation and an increase of 1% per week >20 weeks' gestation. In reporting these trends, both authors suggested the risk of a "no call" falls with advancing gestational age and rises with increasing maternal weight in association with the circulating fetal fraction, but neither study reported the magnitude of these associations.[2,3] Our study was able to describe the magnitude of the association between maternal weight and "no calls" and explore the probability of "no calls" stratified by weight class throughout gestation, providing clinically relevant information that can be used to counsel women in practice.

Our study has many strengths; first, it is generalizable. Our study was performed at a large-volume community-based institution with a variety of clinicians including general obstetrician-gynecologists, midwives, and maternal-fetal medicine specialists. The cfDNA testing included the vast majority of modality options commercially available, and the indication for cfDNA testing varied as it does in the clinical setting. Second, our database, with patient-level data, is well maintained. Our certified genetic counselors prospectively update the database, which keeps missing data at a minimum and avoids many of the systematic problems associated with larger administrative data sets. Finally, our study was able to evaluate a relatively large "no call" cohort that gave us the ability to stratify by obesity class and then evaluate the relationship between "no calls" and maternal BMI over time, as gestational age advanced.

Our study was not without limitation. Our study may be considered "impure" compared to most published investigations of cfDNA because we did not limit our study to a single cfDNA modality or platform. For the patients in our study, the modality of cfDNA was largely dictated by cost, which is influenced by the patient's health care coverage and any charity or rebates the industry may provide. As such, the most affordable modality for any given patient changes frequently and rapidly. Therefore, the utilization of multiple cfDNA modalities and platforms among our population highlights the generalizability of our results and the practical clinical approach of our investigation. Unfortunately, due to limitations within our clinical data, we were unable to evaluate how fetal fraction may impact our results. The fetal fraction is important as maternal weight has been shown to be inversely related to the fetal fraction of circulating cfDNA, but given that cfDNA is a relatively new technology, there are limitations as to our ability to investigate fetal fraction in the clinical setting at this time.[3,5] First, the method for obtaining fetal fraction varies based on the technology used. Second, reporting of fetal fraction has changed over the last several years and fetal fraction is now being reported more robustly by the industry as the importance of fetal fraction has come to light. Third, although the American College of Medical Genetics and Genomics recommends that all laboratories should include a fetal fraction on reports, there are companies that continue to fail to

declare a fetal fraction.[11] Furthermore, while our study is limited by utilizing only a single center, our data were collected in a clinical setting over several years and included a variety of cfDNA modalities that evolved and changed with time and because of this there is no potential for commercial bias. Finally, in a minority of cases, weight was self-reported and although women are weighed frequently during their pregnancy, there is a concern for reporting bias, as women would be expected to underreport their weight. However, Lin et al[12] established women are reliable and accurate at self-reporting their weight.

In conclusion, while we were able to determine the optimal timing for cfDNA sampling that minimizes the probability of "no calls" in obese women, the identified statistically significant cut point of 21 weeks has limited clinical utility as it would limit reproductive choices. However, a clinically significant reduction in the probability of a "no call" from 8-16 weeks for women with a BMI >35 kg/m^2 suggests that delaying cfDNA even a few weeks is a reasonable strategy to reduce the probability of a "no call". We advocate clinicians use this information to inform their decision on timing of sampling and assist in counseling obese pregnant patients on the limitations of cfDNA, with particular consideration regarding the continued important role of traditional serum screening and diagnostic testing. ■

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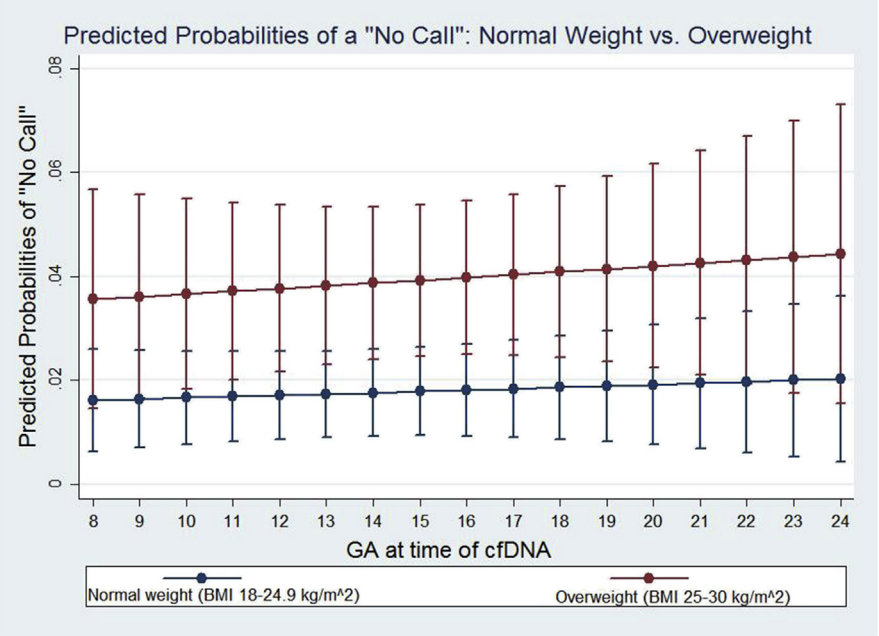
APPENDIX 1
Predicted probabilities of "no call": normal-weight vs overweight women



Graph of gestational age (GA) at time of cell-free DNA (cfDNA) testing vs probability of no call for normal-weight (blue) and overweight (red) women.

BMI, body mass index.

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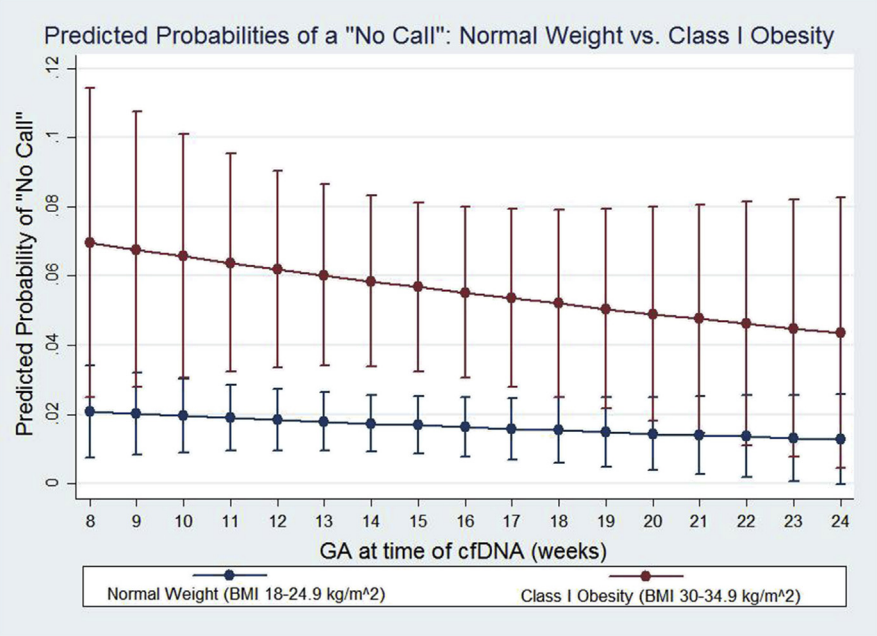
APPENDIX 2
Predicted probabilities of "no call": normal-weight vs obesity class I women



Graph of gestational age (GA) at time of cell-free DNA (cfDNA) testing vs probability of no call for normal-weight (blue) and obesity class I (red) women.

BMI, body mass index.

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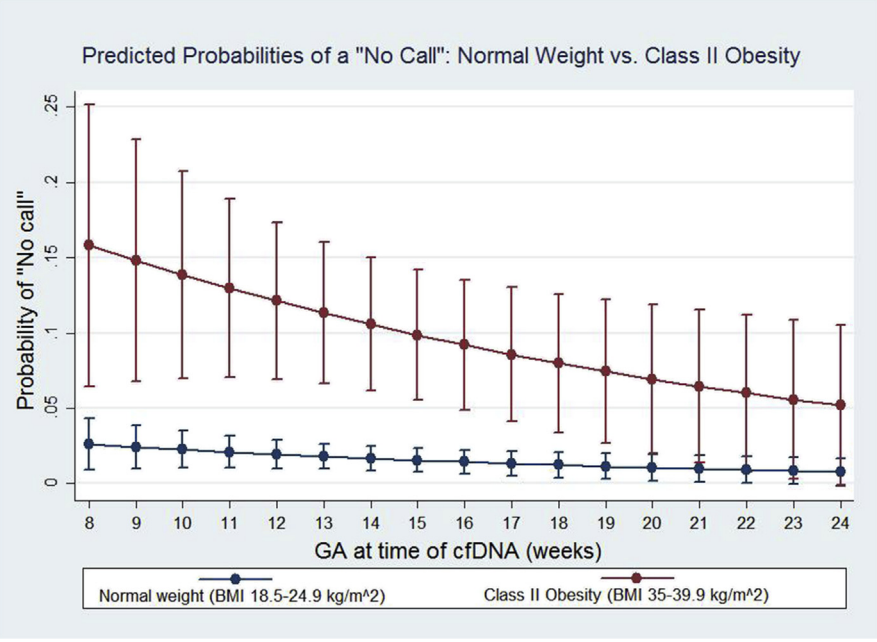
APPENDIX 3

Predicted probabilities of "no call": normal-weight vs obesity class II women



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BMI, body mass index.

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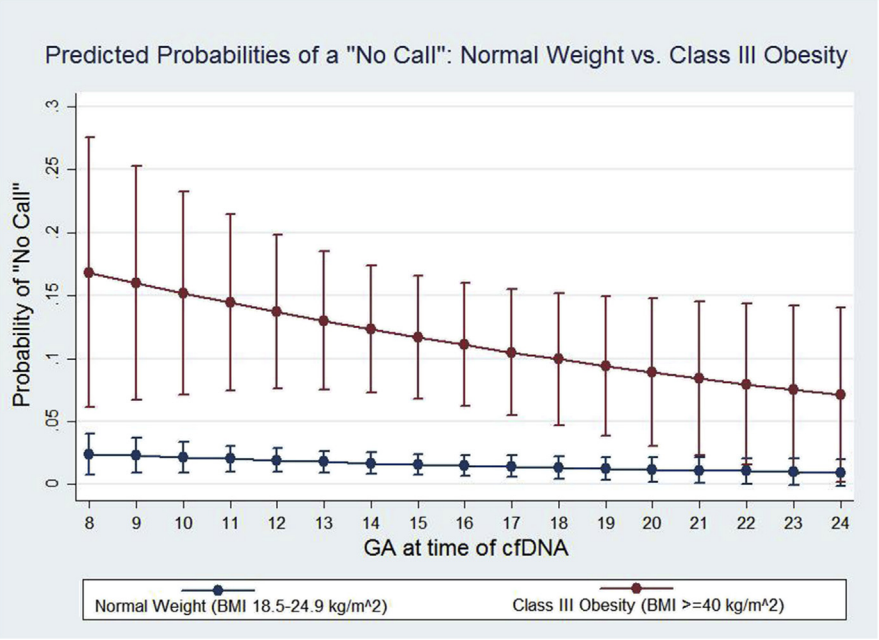
APPENDIX 4
Predicted probabilities of "no call": normal-weight vs obesity class III women



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BMI, body mass index.

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