PROJECT SUMMARY

Since 2004, the *Right from the Start* team has investigated the course and consequences of uterine fibroids in pregnancy. We have found: 1) prevalence of fibroids is higher than previously reported; 2) black women are more likely than white women to have fibroids at a younger age, and to have multiple, and larger fibroids; 3) self-report of fibroid status misclassifies more than 50% of pregnant women; 4) having one or more fibroids is *not* strongly associated with risk of miscarriage or preterm birth. The adjusted hazard for risk of miscarriage or preterm birth comparing women with one or more fibroids to those without are: HR = 0.85 (95%CI: 0.64, 1.13) and HR = 0.90 (95%CI: 0.64, 1.27) respectively. These findings run directly counter to conventional clinical and lay wisdom that fibroids in pregnancy are harmful. The cohort currently includes more than 5,474 community-recruited women with detailed research ultrasounds and prospectively tracked pregnancy outcomes. Mean diameter of a woman's largest fibroid is 2.3 cm (95%CI: 1.8, 2.8) and the majority of women with a fibroid have one. Using mutually exclusive categories: 42% of fibroids are subserousal, 35%, intramural, 17%, submucous, and 5% pedunculated. We are continuing interim recruitment to accrue approximately 900 additional women; however, current protocols will stop obtaining ultrasounds in February 2012. We propose to expand this unique cohort by 2,750 women, to total 9,124. In this phase we will recruit ≥50% women with fibroids in order to assure ample power to extend and refine understanding of what fibroid characteristics (size, type, location, total volume) are associated with adverse reproductive outcomes. Understanding if specific fibroids are associated with harms is of critical clinical and research significance because surgical intervention is common for all types, sizes, and locations of fibroids. The goal of such procedures is to mitigate adverse reproductive outcomes, but we may be targeting the wrong fibroids and using more aggressive approaches than required. If we find certain characteristics are associated with harm (e.g. subserous fibroids increase risk of preterm birth) it may also inform study of mechanisms by which fibroids disrupt normal uterine physiology. We propose to investigate the following primary hypotheses: **H1**: **Specific characteristics of fibroids (type, location, size, total volume) are associated with risk of spontaneous abortion (SAB)** defined as pregnancy loss before 20 completed weeks gestation; and **H2**: **Specific characteristics are associated with risk of spontaneous preterm birth (PTB)** defined as birth following preterm labor and/or preterm premature rupture of membranes prior to term. We will define the related null hypotheses as: no specific characteristic tested has a meaningful association with SAB or PTB, defined as more than a 20% difference in hazard. All the requisite tools for this investigation are in place. We are immediately prepared to pursue these crucial questions about the reproductive consequences of fibroids in pregnancy, which are among priorities identified by federal research agencies including NIH and AHRQ.

Project Narrative

At least 750,000 women with fibroids become pregnant each year in the United States, the majority of which do not know their fibroid status before conceiving and learn they have fibroids at the time of an ultrasound. Studies have reported increased complications associated with fibroids including spontaneous abortions, preterm birth, placental abruption, fetal malpresentation, dysfunctional labor and increased risk of cesarean birth; however, at present, the need for removal of particular fibroid subtypes or for treatment, in an attempt to stabilize or diminish the size of fibroids before pregnancy, is unknown. The objective of our study is to expand our cohort in order to have sufficient power to extend and refine understanding of what specific size, type, and locations of fibroids are the strongest predictors of adverse reproductive outcomes.

SPECIFIC AIMS

In 2004, we launched an unprecedented NIH-funded investigation of the consequences of uterine fibroids in pregnancy. We aimed to determine if fibroids were a plausible cause of miscarriage and/or preterm birth. We had the advantage of prospective enrollment of a large community-recruited sample that overcame many of the biases in earlier literature, as well as universal use of research ultrasound protocols for all participants. We felt confident fibroids were more common than reported in the literature and that we might have an opportunity to explain some of the racial disparities in pregnancy outcomes by taking into account that while adverse events like preterm birth are more common among black as compared to white women in the United States, fibroids are also unequally distributed with black women at higher risk. Our latent objective was to set the stage for intervention trials to compare the effectiveness of novel medical and less invasive surgical approaches for treating fibroids and improving pregnancy outcomes.

Almost 5,500 completed pregnancies later, the *Right from the Start (RFTS): Fibroids in Pregnancy Cohort* is the largest prospective study of its kind. And the plot line is quite different. While fibroids are more than twice as common as previously reported (11%), and more common and numerous among black women[1] they do not appear to be substantive villains in the story of adverse outcomes. Adjusting for covariates with potential to confound the relationships we find:

Table 1. Hazard* associated with uterine fibroids

Compared to not having fibroids	**Miscarriage** aHR (95% Confidence Interval)	**Preterm Birth** aHR (95% Confidence Interval)
≥ 1 Fibroid	**0.85 (0.64, 1.13)**	**0.90 (0.64, 1.27)**
Any subserous	0.64 (0.40, 1.01)	1.20 (0.75, 1.92)
Any intramural	1.08 (0.75, 1.56)	0.79 (0.47, 1.33)
Any submucous	0.99 (0.60, 1.64)	0.82 (0.40, 1.67)

*Cox proportioned hazard ratio approximates relative risk; adjusted for age, parity, race/ethnicity, smoking status, BMI, education, marital status, diabetes status, alcohol use. Miscarriage model also adjusted for caffeine intake.

But do we know the end of the story with sufficient confidence to inform care? **Conventional wisdom encourages intervention for fibroids with the goal of reducing adverse pregnancy outcomes**. Women and care providers believe fibroids are rare but concerning. This is especially true for categories that contact or intrude on the uterine cavity (submucous) or that are numerous or large. In fact fibroids may be common and innocent, except as markers of other risks, or only a liability in very rare instances. This means women may be having unnecessary and potentially harmful interventions that won't deliver the desired results.

Our operational goal is to capitalize on the *Fibroids in Pregnancy Cohort* by building a second phase that oversamples women with fibroids (aiming for 50%, who are likely to have increased severity) and dramatically refine estimates of how fibroid characteristics relate to risk. This would allow us to achieve a total of 9,124 participants (5,474 cohort I, 900 interim ongoing recruitment, 2,750 proposed cohort II). This will provide powerful estimates needed to challenge conventional wisdom and to guide future research.

Specifically, we aim to test the following hypotheses: While our current data suggests presence of uterine fibroids *per se* is unlikely to cause miscarriage or PTB, aggregate estimates may obscure risks associated with specific clinically concerning characteristics such as submucous type or large size. Prematurely ceasing observational research because the overall estimate is null can obscure important influences that might be clinically actionable; thus risking failure to pursue related intervention studies and ultimately under-treating subsets of women who could benefit. On the other hand, expanding this cohort may influence cessation of intervention if evidence is compelling that fibroids are very unlikely to cause harm.

H1 (SAB): Specific characteristics of fibroids (type, location, size, total volume) are associated with risk of miscarriage defined as pregnancy loss before 20 completed weeks gestation.

H0 (SAB): No specific characteristic tested has a meaningful association with risk of miscarriage, with meaningful defined as more than a 20% difference in hazard.

And the companion hypotheses:
H2 (PTB): Specific characteristics of fibroids are associated with risk of spontaneous preterm birth defined as birth following preterm labor and/or preterm premature rupture of membranes prior to term.

H0 (PTB): No specific characteristic tested has a meaningful association with risk of spontaneous preterm birth, defined as more than a 20% difference in hazard.

SIGNIFICANCE

Our work has direct relevance to more than 450,000 US women each year and their care providers who want to know what, if anything, to do about a fibroid in the context of planning a pregnancy or being vigilant during a pregnancy. We are not focused on women with infertility or complex reproductive histories such as recurrent pregnancy loss. We are guided by informing care for an average woman who is contemplating a pregnancy.

Across the reproductive lifespan women are developing fibroids and by menopause, having one or more fibroids is the norm.[44] Evidence suggests incidence rises with age and the curve begins earlier for black women than white. While most women are asymptomatic, imaging, such as ultrasound during pregnancy or MRIs for other reasons, often detects fibroids. Much like the "occulostenotic reflex" in interventional cardiology[1] - see an abnormality, stent an abnormality - the urge to intervene with fibroids can be substantial. In part this is because of a natural, and at times erroneous, belief that correcting anatomic abnormalities corrects or prevents functional abnormalities. Surgical repair of the joint space abnormalities seen in osteoarthritis is a prime example of how restoring normal anatomy does not achieve the desired results.

In the case of fibroids in reproductive age women, the instinct to act is reinforced by three inter-related factors: 1) current state of the science and medical opinion; 2) media and health education materials; 3) desire to take action when adverse events occur. We'll examine each in turn as a means of underscoring the significance of the proposed research for providing critical new information.

To document the **state of the science on fibroids and pregnancy outcomes**, we searched Medline from January 1966 through 2003 using the MeSH headings "pregnancy, explode" and "leiomyomata, explode," restricting this to original research in adult women. This identified 338 publications to screen. We sought studies that included ten or more women with fibroids, included a contemporaneous comparison group, and reported pregnancy outcomes by fibroid status. Twenty-one publications related the presence of fibroids to pregnancy outcomes such as bleeding, miscarriage, preterm birth, and cesarean birth; however, only 12 included a comparison group and reported data to assess spontaneous abortion and/or preterm birth risk. All but one identified participants through clinical care at academic centers; half were exclusively based on women seeking infertility care;[2-7] and all with more than 1,000 subjects were from Italy.[8-10] The majority were restrospective cohorts based on ultrasound databases or case-control studies. None were clearly prospective for all participants. One exception to use of clinical populations was a linkage study based on birth certificate data and hospital discharge data indicating fibroids status based on ICD9 codes, however insufficient data was provided about prematurity risk to be informative.[11]

Table 2. Reported associations of fibroids with spontaneous abortion and preterm birth

Outcome	Studies	Total number of participants		*Summary#*			
		with fibroids* (n)	"without"* (n)	Range of estimates	RR >1	RR ≤1	Meta-estimates RR (95% CI)
Spontaneous Abortion	8	355	3,583	1.3 to 3.0	8/8	0/8	1.6 (1.2, 2.1)
Spontaneous Preterm Births	4	391	8,245	0.9 to 4.2	2/4	2/4	1.7 (0.6, 4.9)^
With preterm labor	5	406	8,368	0.9 to 4.0	2/5	3/5	1.5 (0.7, 3.4)^
With premature rupture of membranes	4	391	8,245	0.6 to 6.0	3/4	1/4	2.1 (0.6, 7.5)^

*Definitions of fibroids and assessment of comparison groups for fibroids varies. ^Random effects models used. #Excludes study with N>12,000 that dominates;[10] meta-estimates of RR including this study are 1.4 and 1.5 for SAB and PTB respectively.

Updating this search from 2003 to present indentified 184 papers to screen. After restricting to studies that reflect a general population of women, we identified no recent studies of fibroids and miscarriage risk (all were done in the setting of fertility care). We also excluded two small studies from procedure databases that examined whether fibroids increased risk of SAB after genetic amniocentesis or chorionic villi sampling.[12, 13] Two studies were additional clinical ultrasound database series[14, 15] and one was additional data linkage study merging vital records with clinical care records.[16] Three new studies that addressed preterm birth had similar findings with effect estimates from 1.0 to 1.75; we calculated a meta-estimate of effect of 1.4 (95%CI: 1.4, 1.5).[14-16] No current publications reflect prospective study of a non-clinical population of women from early in pregnancy. Review of the CRISP database of federal funding confirms no ongoing US cohorts other than *RFTS.*

Based on such evidence, fibroids are blamed for poor reproductive outcomes in popular pregnancy publications and lay health media.[17-35] *What to Expect When You are Expecting*, called the "pregnancy bible for American women" by the New York Times, advises those planning: "Now is the time to get treated for gynecologic conditions that might interfere with pregnancy, including: uterine polyps, fibroids, cysts, or benign tumors."[17] Newsweek notes though "noncancerous, they [fibroids] can make pregnancy difficult, dangerous, and in some cases impossible."[34] Such coverage, often expounding risk of miscarriage, preterm birth, abruption, poor fetal growth, and cesarean, permeates web and print publications as well as televised reports.[17, 31-34]

Adverse pregnancy outcomes are common. Approximately 10% to 15% of identified pregnancies end in loss,[36-38] but 2-3 times this proportion of pregnancies may be lost prior to recognition of the pregnancy.[39] Though direct medical consequences of such losses are usually modest, the emotional impact can be substantial.[40, 41] Known causes of SAB are scant,[37,42,43] with advanced maternal age and a history of spontaneous abortion being the strongest common predictors.[36,37] Cigarette smoking appears to be associated with increased risk,[45] possibly restricted to chromosomally normal losses.[46] Other associations are more speculative including occupational exposures,[47] like solvents,[48-50] glycol ethers,[51] chlorinated hydrocarbons,[52] and anesthetic gases,[53] stressful and physically demanding work,[54] and male occupational exposures.[55] Caffeine in moderate quantities has recently been removed from the list after conduct of large prospective cohorts.[56-59] We are not aware of studies specifically addressing whether SAB risk varies by race or ethnicity but we have a manuscript in preparation indicating black women have a 40% higher risk of loss.[60] PTB, occurring in 12.7% of pregnancies,[61] is the leading cause of perinatal mortality in the US,[62] consuming a large proportion of funds spent for perinatal health care.[63, 64] Efforts to predict impending PTB and delay its occurrence have had limited success;[65-67] so a focus on prevention has been a priority.[68] The strongest predictors of spontaneous preterm birth are a prior PTB and multifetal gestation;[63, 69] neither offers an opportunity for prevention. Other factors associated with risk include maternal age, minority status, genitourinary tract infection, smoking, and low pre-pregnancy weight.[70-75]

Conventional wisdom is an idea or explanation that is generally accepted as true by experts in a field or by the general public or both. Since SAB and PTB are not rare (combined more than 1.14 million events a year in the US), we have the perfect storm – the availability heuristic kicks in and common problems become linked in our explanatory models with a fairly common condition, in this case fibroids. Because no one would want to experience these or other adverse outcomes that have been attributed to fibroids, current challenges for women with fibroids include experiencing anxiety from anticipating complications or acting to remove, ablate, or medically treat fibroids before or between pregnancies. But there are potential adverse consequences related to the intervention. In the case of myomectomy, the most common intervention among women who wish to preserve fertility, harms include hysterectomy for complications at surgery (1 to 4%), blood transfusion (<1 to 21%), infection (2 to 4%), potential for adhesions and impaired fertility, recurrence of fibroids as high as 62% over three to four years, and near certainty of a scheduled cesarean since the myomectomy scar weakens the uterus and women are advised not to labor in subsequent pregnancies.[76] Given the current state of the literature, women and clinicians lack high quality information to fully understand the magnitude and absolute risk of adverse outcomes associated with having a fibroid. This is required in order to weigh whether it is worthwhile to attempt to reduce risk through intervention. In the absence of trials or national surgical registries, population-based epidemiology with robust adjustment for factors that could bias results is a viable option. Building on the ***RFTS: Fibroids in Pregnancy Cohort*** **is a timely and efficient means to get better answers and to establish a unique data resource** reflecting more than 9,000 participants that can be shared and explored by investigators for years to come.

INNOVATION

As described, epidemiologic and medical knowledge about the implications of fibroids in pregnancy is insufficient. The literature about how fibroids and fibroid characteristics influence pregnancy is meager, and in most areas conflicting. Most is based on subspecialty referral populations that don't provide relevant information for the typical woman or her care provider. Federal research agencies including the NIH NICHD and AHRQ recognize fibroids among their priority women's health topics because tens of millions of women in the United States have fibroids and answers remain elusive.

A search of the CRISP database of federally funded research does not identify any other studies investigating uterine fibroids as they relate to adverse pregnancy outcomes. Three reproductive epidemiology studies list fibroids detected by clinical ultrasound as a covariate of interest but none have early prospective enrollment

with use of standardized research ultrasounds to both evaluate miscarriage and later pregnancy events. They also lack deep, high quality data that is collected in a fashion to allow inclusion of important covariates (such as very early pregnancy BMI, alcohol consumption and smoking behavior and changes in habits, prescription and over-the-counter medications, onset of prenatal/multivitamin use, income, education, and employment).

If the effects linking fibroids to adverse outcomes that are seen in smaller studies are erroneous, the error likely results from methodologic issues described, poor statistical precision, and inability to adjust for important confounders. If we do not adjust for key confounders in data from the current *RFTS* cohort, the estimates of risk of SAB from having a fibroid compared to none is 1.4 (95% CI: 1.0, 1.8) and for PTB is 1.2 (1.0, 1.5). Capacity to thoroughly assess confounding, using advanced time-to-event modeling methods that allow for varying time at risk and for time-varying covariates, is required to obtain reliable estimates. The quality and depth of the data combined with advanced quantitative methods will be a new and crucial contribution of the *Fibroids in Pregnancy Cohort* to knowledge about fibroids.

NIH evaluation criteria for innovation speak directly to the value of shifting "current research or clinical practice paradigms" using novel theoretical concepts, approaches or methodologies. Relatively neglected areas may be at a disadvantage if we don't recognize the importance of laying the correct foundation. If such a foundation is missing, as it is in research on fibroids and reproduction, then the novelty and value of a large, community-recruited prospective cohort is immense. With the addition of 2,750 women, oversampled for having fibroids, the *Fibroids in Pregnancy Cohort* will be the most powerful tool we have to examine the influence of fibroids and their characteristics on pregnancy outcomes in the general US population. At its current size this cohort is the largest and only study of its kind. But our data is insufficient to drive a shift in current research and clinical practice paradigms because of the precision of the estimates we can provide. Despite our initial assumptions when we planned the cohort, we find (and simulation models presented with the power calculations below support) that our results are astonishingly null in the face of conventional wisdom:

> "...why is it whenever a woman is diagnosed with fibroids her ability to have children is called into question or she is advised to become pregnant sooner rather than later? ...research suggests that fibroids can impair a woman's ability to conceive or cause miscarriage or preterm labor." - *It's a Sistah Thing*[30]
>
> "...most experts in the field of reproductive endocrinology believe the fibroid must be about the size of a golf ball (or above 3 centimeters) for it to have an adverse effect on fertility or pregnancy. Most physicians agree that even smaller fibroids should be removed if they are distorting the endometrial cavity, and that all fibroids the size of a baseball (greater than 6 centimeters) should be removed regardless of their position." - *Making a Baby*[23]
>
> "Other causes of late miscarriages are abnormal attachment of the placenta, uterine fibroids, and incompetent cervix, or infections." - *The Pregnancy Book*[29]
>
> Preterm labor has many possible causes including "uterine anomalies such as large fibroids, which decrease the ability of the uterus to stretch and accommodate the growing baby." - *Your Pregnancy: Week by Week*[24]

Medical texts and expert reviews generally align with the lay literature (much of which is written by physicians):

> "Placental implantation over or in contact with a myoma increases the likelihood of placental abruption, abortion, preterm labor, and post-partum hemorrhage." - *Williams Obstetrics*[77]
>
> "[Women]... who wish to preserve fertility should have a myomectomy." - *Management of Common Problems in Obstetrics and Gynecology*[78]

The *Fibroids in Pregnancy Cohort* will provide crucial refinements of what we know and will likely land the field squarely in territory that Mark Twain described: "It ain't what you don't know that gets you into trouble. It's what you know for sure that just ain't so." Increasing confidence that fibroids are not *per se* a risk is valuable as is identifying specific types or sizes as good candidates for intervention. Both potential outcomes of our work would have exceptional value by better targeting care and refining intervention studies, or by reducing unnecessary intervention and worry. Latin for innovation has the meaning "to renew or change"; RFTS fibroids data is well along the path to changing how we understand the role of fibroids in pregnancy. The proposed expansion of the cohort will speed us along.

APPROACH

Overview of RFTS: Fibroids in Pregnancy Cohort II

The proposed prospective study will add 2,750 women with a high prevalence of fibroids, to complete a cohort of 9,124 women (Table 3) to examine the consequences and course of fibroids in pregnancy, with emphasis on understanding the specific contribution of fibroid characteristics to pregnancy outcome.

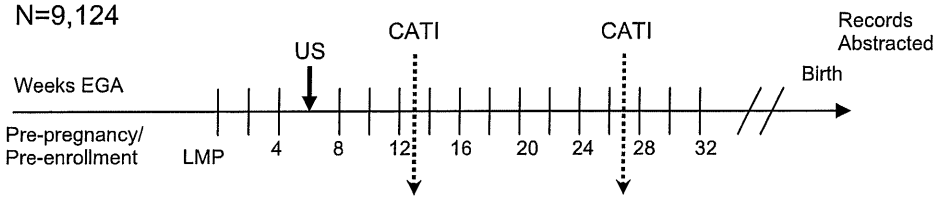
Table 3. Estimated participants with fibroids and primary outcomes in completed cohort

	Participants	With ≥ 1 fibroid n (%)	SAB	Preterm Birth
Fibroids in Pregnancy I	5,474	596 (11)	502	363
Interim Enrollment*	900	225 (25)	87	60
Fibroids in Pregnancy II*	2,750	1,375 (50)	289	183
Total	**9,124**	**2,196** (24)	**878**	**606**

*Based on recent and projected modifications of recruitment strategy and current event risk for women in the cohort.

We will enroll women who are less than 9 weeks pregnant, or who are currently trying to conceive (pre-enrollment). Participants will be screened by telephone and an adaptive sampling algorithm will be used to achieve our target of 50% women with fibroids. The algorithm will hinge on knowledge of fibroid status – when the woman was told she has a fibroid and how she reports being assessed. After screening and selection, a research assistant will meet participants to document a positive pregnancy test and obtain informed consent. A study ultrasound (US) will be targeted for the sixth week of gestation, and not later than 10 weeks (Figure 1). Our core survey instrument with items added about fibroid diagnoses and treatment will be administered by computer assisted telephone interview (CATI) at the close of the first trimester. In this interview we obtain maternal health, reproductive, diet, weight, occupational, and behavioral data. In the 27th week we will obtain a pregnancy update from participants including confirmation of care and birth site, any history of bleeding, and any new medications or change in selected behaviors. For all participants, a focused abstraction of clinic and hospital records will be done at the conclusion of pregnancy. Study methods and core data are compatible across all phases of RFTS and the full cohort of 9,124 will be used to examine the association between fibroid characteristics and risk of SAB and spontaneous preterm birth.

Fig 1. The Fibroids in Pregnancy Cohort: Parts I and II
N=9,124



Study Population:

Since January 2001, RFTS has enrolled women from seven metropolitan areas: Raleigh (1,036), Durham-Chapel Hill (2,206), NC; Chattanooga (15), Knoxville (19), Memphis (816) and Nashville (970), TN; and Galveston, TX (411). For the proposed *Fibroids in Pregnancy Cohort II,* we will amplify recruitment in Tennessee continuing to use the name *Right from the Start: A Study of Early Pregnancy Health* as the community marketing title and the public face of the study. This name with a general and positive tone, discourages enrollment based on perceived high-risk status and is familiar in the recruitment areas with strong word-of-mouth referrals and "brand recognition."

For recruitment we will use materials with a general early pregnancy research theme and other materials which focus on attracting women with fibroids who are planning or in early pregnancy. We will use four proven approaches: 1) advertisement in venues and publications with female patrons; 2) direct mailing and pregnancy test coupons introducing the study; 3) referrals from community prenatal care sites (public and private; none are tertiary care); and 4) church and community organization outreach (Appendix). Calls are routed to a centralized toll-free line. During the period of the proposed study we will get side-stream benefits from advertising for the National Children's Study (NCS) which will be running concurrently (Hartmann, PI). Since NCS selects participants from a small, predetermined segment of the population, we have high flow-through of

women who call and are not NCS eligible. NCS ineligible women will be screened during the same call for *RFTS: Fibroids in Pregnancy* creating synergy between the outreach staff and recruitment budgets.

Screening and Enrollment

Our toll-free number is on all pregnancy study recruitment materials. Interested women who are pregnant or planning to conceive are screened by telephone for eligibility (Table 4). For those planning a pregnancy, up to six months of free home pregnancy tests are provided. Final eligibility is established when pregnancy is documented. We predict the volume of calls from eligible women will exceed our enrollment targets by 3 to 5-fold, thus we will be able to implement a fibroid-based sampling system within the computerized screening system. Study staff will enter responses to eligibility questions, including fibroid status and LMP, and also gather basic demographic information. An adaptive sampling strategy will be used that favors early gestational age and targets a minimum enrollment of 50% women with fibroids. We have found that 65% of women who report they have been told they have a fibroid, have a fibroid detected on ultrasound (many are told they may have a fibroid based on bleeding symptoms and not imaging). Among those who believe they do not have a fibroid, just fewer than 10% do. This means we can select almost exclusively for women who believe they have a fibroid, knowing many will not and can become part of the comparison group. The smallest subset will be 5 to 10% recruitment of women who have never been told they have a fibroid.

Table 4. Inclusion criteria for the *Fibroids in Pregnancy Cohort*

- No use of assisted reproductive technology to conceive
- Elementary English skills
- Willingness to have ultrasound
- Willingness to release records
- Intention to be in the area over 18 mos.
- Maternal age ≥ 18 years
- Enrollment by ≤ 9 6/7 weeks EGA
- Intention to carry to term

To achieve our targets, the model will be programmed to over-select from those who have been told they have fibroids; we will monitor the proportion of fibroids we are achieving in real-time and adjust the algorithm as needed to maintain targets.[79] Prior pregnancy outcome will not be used as a screening criterion. Screening staff will not be able to influence sampling; women not selected will be offered other studies. Once the proposed study is underway, the stochastic model for sampling will be fully adaptive, based on characteristics of the call population.[80] Back calculation to the original sample of callers will always be possible enabling us to regenerate the entire sampling process for descriptive purposes.

To assure ultrasound in the proper window, the ultrasound visit will be scheduled once eligibility, selection, and desire to enroll is established, if possible while the participant remains on the telephone. Prior to the ultrasound, study staff will visit eligible women to provide additional information about the study, document a positive pregnancy test, and obtain written informed consent.

Data Collection (in usual order of collection)

Intake/Baseline: For eligible women, basic demographic data and contact information are entered in the tracking system during the screening and baseline call (Appendix). Date of first positive pregnancy test, LMP, last contraceptive method, and certainty about LMP and/or timing of conception, as well as smoking status and medicines are also recorded during this initial phone intake.

Ultrasound: An endovaginal ultrasound (standard for first trimester imaging) will be scheduled, ideally for the week between the 6th and 7th week of gestation; not later than the 10th week. To assure convenience, appointments are offered before, during, and after work hours, and on Saturdays. We will continue to send a courtesy copy of all study ultrasounds to the prenatal care provider designated by the participant. Women who have losses prior to their scheduled research ultrasound are invited to return for a study ultrasound within one month of the loss, ideally within two weeks in order to document the presence or absence of fibroids. In our experience, having had a loss, these participants are eager for additional information and nearly all are willing to have an ultrasound. For data analysis and follow-up scheduling, gestational age will be assigned based on the ultrasound unless the gestation was non-viable at the time of ultrasound in which case LMP dates will be retained. Preference for ultrasound dates is based on reported standard deviation of within 3 days from known dates of conception/implantation; this is substantially more accurate than the deviation based on self-report even with "known" LMP.[81, 82]

Ultrasound Standardization: We have experienced teams of two to three research sonographers conducting studies in Nashville, Chattanooga, and Knoxville, TN. Dr. Glynis Sacks (co-investigator), Director of Women's Imaging at Vanderbilt, and a nationally recognized expert in pelvic sonography, will continue to oversee ultrasound training and protocols to assure the highest level of standardization and reliability possible.

Sonographers have a minimum of five years experience in pelvic sonography including obstetric scanning and current ARDMS certification. All participate in calibration and reliability testing as well as annual training retreats. To assure machines are precisely and identically calibrated, we will use standard phantom targets down to 1 mm to test the endovaginal and abdominal probes. Validation work will be conducted across the size spectrum emphasizing the bounds of our interest (min. diameter = 5 mm), with a fibroid phantom. Training goals are within 0.1 cc volume agreement with themselves and each other. This is consistent with the sonography literature, and our sonographers will likely sustain greater reliability.[83, 84] This testing is repeated annually and measures of agreement will be used to estimate the variability in study measures. Thus, we will ensure that the measures will be of good quality for ranking the size of fibroids and for establishing quantiles defined by volume or other measures such as maximum diameter, for use in analysis.

Table 5. Content of First Trimester (6-7 week) Ultrasound Assessment

- Fetal Viability and Dating
- Uterine Dimensions
- Presence & Location of Fibroids
- Size of Fibroids
- 3-D Volume of Fibroids
- Presence of Fibroid Feeder Vessel(s)
- Resistance Index of Feeder Vessel(s)
- Uterine & Umbilical Artery Velocimetry
- Location of Placenta

Quality of ultrasound is most at risk from missed fibroids or by failure to uniformly classify fibroids. This is directly related to time required to do a systematic study, to uniform training, and to consistent use of quality assurance techniques. Therefore, we supplement our ultrasound report forms and still photos with real time CD/DVD recording of a slow longitudinal and transverse survey of the uterus, as well as still-image recording of caliper placement and measures, for all scans. Each fibroid has three perpendicular diameters recorded in three repeated measures (nine measurements total) – in other words, the sonographer "unfreezes" the image, relocates the target and measures the fibroid three separate times recording still images and caliper marking to CD for each of the repeated measures. We also record the machine-generated averages of the three separate measures for data entry confirmation. Prior to coding and batching for data entry, ultrasound forms are checked for completeness and errors. All abnormal ultrasounds, inconsistencies, dating discrepancies of greater than one week, and potential errors based on range checks are reviewed by Dr. Hartmann. In addition, she reviews the forms, CD images, and coding of all ultrasounds with fibroids noted, prior to data entry. This is the mechanism by which rapid confirmation of the presence of fibroids will be obtained. Quarterly, she and Dr. Sacks will complete re-review of ultrasound data summaries, still photos from all ultrasounds, and a random 10% sample of CD/DVD images from those assessed as without fibroids.

Interviews: An extensive CATI is conducted at the end of the **first trimester**, not later than 15 weeks **gestation**. Content includes detailed maternal health, weight, and reproductive history; maternal health behaviors including smoking, alcohol, caffeine consumption and recent dietary and physical activity patterns; early pregnancy events and symptoms; and paternal data (Appendix). Based on the current interview, we project the average interview will take 45 minutes. A follow-up CATI with two versions - one for those who are no longer pregnant and one for those who are - will be conducted between 26 and 28 weeks EGA. It will take roughly 15 minutes for those with uncomplicated pregnancies and 5-10 minutes longer for those who have had SABs or complications such as bleeding. This interview also provides an additional opportunity to confirm prenatal care and planned birth site.

Ascertainment of Pregnancy Outcomes and Site of Care for Birth/Loss: Participants are encouraged to call the study staff if they have an SAB, therapeutic abortion, or will be leaving the study area. To date we have documentation of pregnancy status through the 20th week for 98% of women who have passed the 20 week mark, including 16 women who reported pregnancy terminations. Of the women who have had SABs, the majority either notified study staff directly or were identified at the time of baseline US. Few were reported at follow-up CATI. For the proposed study we will continue to provide encouragement for participants to contact study staff with updates. For women who have had losses, medical records from the sites that a woman reports were involved in her care (clinic, ER, etc.) are abstracted as quickly as feasible. Follow-up CATI and medical record review for all participants helps assure uniform opportunity to inquire about and document pregnancy loss, independent of individual reporting. We will continue to use these "fail-safe" opportunities. Additional detail about abstracting is provided below. We further enhance our ability to document both losses and births by providing an active reporting incentive for notifying the study office of pregnancy outcome. At enrollment, participants receive a one-page form and postage-paid envelope to return at the conclusion of their pregnancy. The form confirms their address, prenatal care site, and delivery site, or site of care for SAB. Upon receipt in the study office they receive a small gift or $5. A study newsletter is mailed each Spring (Appendix); this provides another method of indirectly checking the quality of our participant contact logs. If more than 4

weeks pass between EDC and receipt of the form we will call the participant to obtain the information by phone and/or mail another form. At any time if we believe we have incorrect phone or address information we use web-based "people locator" tools, including those driven by Social Security Number; and, if necessary contact the individual(s) whose name was provided by the participant as an alternate contact.

Outcome Assessment: Prenatal and Hospital Record Abstracts: Medical charts are abstracted for all participants at the end of pregnancy, whether it ends in a SAB, therapeutic abortion (if any prenatal care was received), stillbirth, or live birth (Appendix). At recruitment, consent is obtained to review all records pertaining to the study pregnancy, and request for medical records release forms will be obtained. For pregnancies that end in spontaneous abortion, we request records from the prenatal care provider or other health care site, such as a walk-in clinic or emergency room, where the woman reported receiving care. Information about where participants sought care for complications is specifically requested in the modified CATI for those women with losses between enrollment and interview.

We focus abstracting on information about prenatal care, including laboratory tests for genital tract and urinary tract infection, blood pressure, diabetes, history of pain/degeneration of fibroids, other complications of pregnancy, and medications prescribed during pregnancy. For SABs, we abstract the components of the prenatal record information that is available and seek reports of pathology examinations, ultrasounds, or other procedures done to characterize the pregnancy loss. For live births, once the prenatal record abstract is completed we also review the hospital chart. We collect data on history at the time of admission, onset of contractions, time of rupture of membranes, fetal presentation, mode of delivery, birth weight, congenital anomalies, and complications. This enables us to categorize preterm births and to facilitate eventual secondary analyses of outcomes such as malpresentation, abruption, emergent cesarean, retained placenta, and post-partum hemorrhage.

Data Management

The complexity of RFTS requires rigorous data management and quality control procedures that will be sustained in *Fibroids in Pregnancy Cohort* data collection. Confidentiality of all data is our highest priority; redundant non-personal identifiers allow us to assure proper linkage across data elements. All data entry platforms, including the tracking system and computerized medical record abstracting program include range checks and assessment of completeness and consistency across items. The data entry and computing staff engage in several levels of error detection, correction, and routine summary reporting. Soft checks prompt immediate re-review of values and hard checks prevent entry of questionable values until the data is flagged and verification is obtained. Standard data reports are presented at monthly study meetings; new summaries and reports are developed as needed to track specific process improvement and efficiency goals. Because we have piloted the new components required for this proposal, we are confident that necessary additional data management tools are in place.

General Analytic Approach

Data analysis will proceed from univariate descriptive analysis to bivariate contingency tables so that the associations among variables, and within and between strata, are carefully characterized prior to multivariable modeling. Before investigating the relationship of variables to outcomes, we will inspect the distributions of continuous variables across the full range of values. During bivariate analysis we will use spline regression and transformation as necessary to help establish cut-points and to identify discontinuities to be taken into account in categorical analyses. Our approach to investigation of our primary hypotheses and related secondary analyses is outlined below by outcome. In each case we will progress from influence of having one or more fibroids to influence of specific fibroid characteristics and then to secondary analyses.

Primary Hypotheses and Secondary Analyses about SAB

H1 (SAB): Specific characteristics of fibroids (type, location, size, total volume) are associated with risk of miscarriage defined as pregnancy loss before 20 completed weeks gestation.

H0 (SAB): No specific characteristic tested has a meaningful association with risk of miscarriage, with meaningful defined as more than a 20% difference in hazard.

Fibroids and Risk of SAB: Our first step will be to obtain an unbiased estimate of the overall relationship between presence of fibroids (present vs. absent) and SAB risk; we will then proceed to examine characteristics. Initially risk of SAB, by fibroid status (Yes/No), will be calculated as the number of pregnancies ending in SAB divided by the total number of pregnancies, including live births, stillbirths, therapeutic abortions, ectopic and molar pregnancies. Risk ratios will be calculated comparing those with and without fibroids, as well

as between strata of fibroid characteristics, such as any submucosal fibroid vs. none and any fibroids in the top quartile of size vs. smaller/none. These calculations will be made largely for comparison to other studies that have less detailed information available, as this approach does not optimize use of data about time at risk.

A more useful index of risk takes into account variable time under observation – some women will enroll within days of a positive pregnancy test, others as late as the 9th week of gestation. A small number will elect to have abortions, truncating time at risk for loss. To the extent that entry and exit times are nonrandom, there is potential for bias across gestational age and between women with and without fibroids.[85] The screening call at which eligibility was determined will serve as date of entry. We will use life-tables to calculate conditional probabilities of the pregnancy ending or surviving by one-week intervals between weeks 4 and 20.[86] This window was chosen because 4 weeks from LMP is the earliest this study can confidently identify pregnancies and after 20 weeks losses will be considered stillbirths/preterm births. Women enter the analysis when they enter observation, (in the week of gestation in which they enroll) and contribute time under observation until they pass beyond 20 weeks, experience an SAB, or are censored (e.g., due to therapeutic abortion). The cumulative risk of spontaneous abortion is the sum of the unconditional failure probabilities over the period of weeks 4 through 20 EGA. Cumulative life-table risks will be calculated by fibroid status and risk ratios will be assessed to compare groups.

To extend the life-table approach to handle continuous exposure measures, allow for time-varying characteristics (such as change in medications or smoking habits), and adjust for potential confounding factors, we will fit appropriate survival models such as Cox proportional hazard models or accelerated failure time models. These models will provide adjusted hazard ratios for the independent influence of having fibroids. The list of potential confounders is limited by the paucity of known or strongly suspected risk factors for SAB. As the cohort grows, we will be able to assess the effects of age (finely stratified given the association with advancing maternal age), race/ethnicity, tobacco use (and changes in use), parity, BMI, comorbidities including diabetes type and hypertension, education, race/ethnicity, marital status, alcohol use (and changes), caffeine use (and changes), prenatal/multivitamin use, prescription and over-the counter medications including non-steroidal anti-inflammatory drugs. The proportional hazard assumption for each potential confounder will be assessed with log cumulative hazard graphs. The full hazard model will be developed using backward elimination with assessment of likelihood ratio tests at each iteration. We will evaluate effect modification for any candidates that are strongly predictive of SAB but not likely to act as true confounders.[62]

Fibroid Characteristics: We will operationalize fibroid characteristics based on the first trimester ultrasound (a sub-cohort of *Fibroids in Pregnancy Cohort I* had repeated ultrasounds). This allows us to retain the entire cohort in analysis. Once the survival models are established for assessing the influence of fibroids on SAB as described above, we will examine specific fibroid characteristics: type (submucous, intramural, subserous, pedunculated/other), number, size (mean diameter), and volume (total volume of all fibroids). We use mutually exclusive definitions of type for each fibroid. Those distorting the endometrium and those directly in contact without identifiable myometrium between the fibroid and the cavity are coded as two categories of submucous, regardless of whether they have intramural or sunstroke components. Those that distort the external contour of the uterus are coded as subscribe. Fibroids that neither contact the cavity nor distort the surface are coded as intramural, anterior, posterior, right, left, and uterine segment are also coded as location and segment for analysis.

To address size, we will conduct analysis at the level of individual women and select the fibroid for the analysis in two fashions: 1) using the fibroid with the largest mean diameter (average of the repeated measures of the perpendicular diameters) and 2) randomly selecting which fibroid to contribute to the analysis of size, with repeated random selection and reanalysis. Assessing influence of size in this fashion is a precaution since more than 75% of women will have only one fibroid.

Inspection of distributions of fibroid diameter and volume (for an ellipsoid = D1 x D2 x D3 x 0.534) together with bivariate analysis and insights from study of natural history in the prior subcohort will assist us in selecting the form in which size enters multivariable models (i.e., as a continuous or categorical variable), and guide categorization into statistically meaningful quantiles as needed. **Our intention will be to assess association of each fibroid characteristic and risk of SAB.** We will then extend the models to address type and location while also adjusting for size or volume, and conversely examine the influence of size or volume adjusted for location and type. The large proposed "N" for the cohort makes such detailed nested models possible.

Secondary Analyses: The proposed expansion of the cohort will also allow us to further **characterize subsets of spontaneous abortion** using US data to divide cases based on developmental stage documented

prior to loss. Losses with US findings consistent with anembryonic gestations will more often be due to genetic abnormalities compared to losses with fetal measures and heart rate consistent with normally progressing gestation. The expectation for this sub-analysis would be that if fibroids exercise a purely architectural influence on risk that is obscured by the 32% prevalence of anembryonic losses among all losses, then risk of anembryonic losses should be similar across women with and without fibroids while loss of "normal" gestations would be increased among women with fibroids. In contrast, if fibroids exert influence through impairing the quality of implantation and/or promoting inflammatory processes, we might see the converse. We will also analyze spontaneous abortions by timing of occurrence; i.e., risk up to 12 weeks and risk beyond 12 weeks EGA. We will also use data from Doppler obtained for women with fibroids to assess if a fibroid lies in a perpendicular plane beneath the area of implantation. We will evaluate whether SAB (or PTB) risk is increased by the **presence of sub-placental fibroids.**

Primary Hypotheses and Secondary Analyses about PTB

H2 (PTB): Specific characteristics of fibroids are associated with risk of spontaneous preterm birth defined as birth following preterm labor and/or preterm premature rupture of membranes prior to term.

H0 (PTB): No specific characteristic tested has a meaningful association with risk of spontaneous preterm birth, defined as more than a 20% difference in hazard.

Fibroids and Risk of PTB: As described in our general approach to analysis, we will first describe the relationship between fibroid presence and PTB in bivariate and stratified analyses. Gestational age will be assigned based on first trimester ultrasound (algorithm described earlier). Births before 37 weeks gestation will be classified as: 1) **preterm labor** defined as spontaneous labor and delivery; 2) **preterm premature rupture of membranes** defined as rupture of membranes documented to have occurred ≥ 4 hours before onset of regular uterine contractions; and 3) **medically indicated** including those for abruption, bleeding previa, pre-eclampsia, or other maternal or fetal indication. Medically indicated preterm births will be described by fibroid status but will be excluded from multivariable models. Based on literature review the following will be considered *a priori* candidate confounders of the relationship between fibroids and spontaneous PTB: maternal age, race/ethnicity, income, access to insurance, education, parity, pre-pregnancy weight, and smoking status. Some markers such as bleeding and history of pain in pregnancy may be in the causal pathway or closely tied to presence and/or characteristics of fibroids, but not be independent risk factors. Other predictors, such as prior PTB, may well influence the association but not constitute a true confounder.[87] These factors will be examined and described; however, they will not automatically be included in multivariable models for adjustment. Preliminary assessment of potential to confound will be conducted by evaluating the association between the candidate confounder and risk of PTB among those without fibroids, and the relationship between the presence of fibroids and the candidate. Factors related both to PTB and to fibroids, even weakly, will be included in the next phase of analysis.

We will construct logistic models for spontaneous preterm birth < 37 weeks and for birth ≤ 34 weeks, with the principal goal of obtaining an unconfounded estimate of the risk of PTB associated with presence of uterine fibroids. Fibroid status and all candidate confounders will be included in the initial model that will then be reduced. Each variable will be considered in a full model adjusted for the other candidates. Variables that have $p \leq 0.25$ for the likelihood ratio test or result in a 10% or greater change in the point estimate for the odds ratio of PTB given presence of fibroids will be retained. When all such confounders are identified, they will enter a final logistic model to estimate the independent effect of fibroids on PTB risk. These models are created for comparison to existing literature that employs logistic models.

After construction of standard logistic models, we will employ Cox models or accelerated failure time models. We will use these to generate the interval and cumulative hazard ratios that relate presence and characteristics of fibroids to risk of PTB using the analysis approach described in detail in the SAB analysis plan above.

Secondary Analyses: We will also construct Kaplan-Meier survival curves to portray the time-to-delivery among women with and without fibroids and by fibroid characteristic as indicated. This approach will shed light on whether or not the distribution of EGA at birth is shifted or differently distributed among women with fibroids and PTB.

Study Size and Statistical Power

We have approached estimation of likely study population characteristics and statistical power through use of simulation models. Table 6 below provides a summary of the probable fibroid characteristics of the **full cohort of 9,124 women (5,474 cohort I, 900 interim ongoing recruitment, 2,750 proposed cohort II)** based on the

characteristics of members of the *Fibroids in Pregnancy Cohort I*, described in the first row of fibroid descriptive data below. The estimates for Interim and *Fibroids in Pregnancy II* characteristics draw on the answers to existing interview items about knowledge of fibroid status (which will be used to screen participants for the proposed new phase), prior fibroid surgery, and demographic predictors.

Table 6. Estimated fibroids profile among women with one or more fibroids (n=2,196 with fibroids)

From among an estimated 2,196 women with at least one fibroid (see Table 3).	With ≥ 2 fibroids n (%)	Mean diameter largest mm±sd	Mean total fibroid volume mm±sd	Any Sub-serous n (%)	Any Intramural n (%)	Any Sub-mucous n (%)	Any Other n (%)
Fibroids in Pregnancy I	169	23.0±16.4	27.7±79.2	241 (40)	270 (45)	126 (21)	46 (8)
Interim Enrollment	75	23.8±17.1	33.2±90.8	93 (41)	105 (47)	49 (22)	19 (8)
Fibroids in Pregnancy II	630	26.1±18.8	49.6±117.5	604 (44)	694 (51)	334 (24)	141 (10)
Total	**874**	**25.0±17.9**	**41.9±105.5**	**938 (43)**	**1,069 (49)**	**509 (23)**	**206 (9)**

Because conventional power calculation tools do not adequately capture the covariance between complex webs of inter-related factors (e.g., age, BMI, smoking, prior pregnancy outcomes, diabetes type and status, alcohol use) and those that adjust for R-squared can only do so in a generalized fashion, we undertook simulations to allow the complexities of observed associations to be captured in estimating the most probable estimates of effect and the precision/power of those estimates, in each instance using a two-sided $\alpha = 0.05$.

The current sample of 5,474 was oversampled using a bootstrapping approach. For each of 1,000 independent runs conducted for each estimate, 900 additional women with a 25% chance of having fibroids and 2,750 additional women with a 50% chance of having fibroids were added to the current cohort. The number of additional women added with fibroids was generated as the sum of two independent binomial random variables, and each was equally likely to replicate any of the 596 women in the current cohort with fibroids. The remaining additional women without fibroids were equally likely to replicate any of the 4,878 women in the current cohort without fibroids. For each run, the Cox proportional hazards model, accounting for multiplicities, was run with the full simulated population of 9,124 women. The parameter estimates and standard errors from the 1,000 runs were averaged to construct overall estimates and confidence intervals, and the fraction of runs whose confidence intervals did not include 1.0 was used to estimate the power for each parameter.

Table 7. Simulation of hazard associated with uterine fibroids and anticipated power

Compared to not having fibroids	SAB		PTB	
	aHR (95% CI)	Percent CIs projected to exclude HR = 1	aHR (95% CI)	Percent CIs projected to exclude HR = 1
≥ 1 Fibroid	**0.85 (0.72, 0.99)**	**0.50**	**0.93 (0.77, 1.12)**	**0.06**
Any subserous	0.63 (0.50, 0.81)	0.99	1.22 (0.97, 1.55)	0.38
Any intramural	1.07 (0.88, 1.31)	0.07	0.80 (0.61, 1.04)	0.35
Any submucous	0.97 (0.74, 1.27)	0.02	0.80 (0.56, 1.14)	0.17

*Includes age, parity, race/ethnicity, smoking status, BMI, education, marital status, diabetes status, alcohol use. Miscarriage model also adjusted for caffeine intake.

The table above illustrates our situation well: overall we are not in most instances in the position of hoping to confirm an effect; we are aiming at maximizing the precision of a null estimate in order to best be able to bring into question the wisdom of intervening when the effect is null or protective. A larger cohort affords greater opportunity to be confident that the influence of having most types of fibroids is not harmful, i.e. that conventional wisdom is in error. If effect sizes are larger for some of the fibroid characteristics (for instance subserous), or the severity of disease by size, volume, or number of fibroids is greater than we expect, we will have greater power to detect a difference.

Strength of the Research Strategy and Solutions to Potential Challenges

We have demonstrated our ability to acquire the required data and can continue to do so without disruption. (Though without additional funding our current protocols will **stop obtaining ultrasounds in February 2012**.) Central challenges in community-based recruitment and engagement of care providers have been met, as

have development of tools like data management systems and interview templates, and building an experienced staff. Our team of epidemiologists, biostatistician, sonography experts, colleagues at NIEHS, and graduate students are eager to press forward for definitive answers. We feel that very strong data will be required to begin to question conventional wisdom and to assure that all women and providers have the information they need for informed decision making about fibroids during the reproductive years.

We are confident that prospective investigation of the spectrum of adverse reproductive outcomes – that will naturally extend past the grant aims to include others like cesarean risk and growth restriction – must begin in very early gestation, and be done in a racially and ethnically diverse population. This strength of our study design sets us apart from others. Use of analytic methods that incorporate assessment of risk as a function of time will also advance the sophistication of consideration of questions about fibroids in pregnancy.

The first *RFTS: Fibroids in Pregnancy Cohort* was motivated in part by a goal to understand the true prevalence of fibroids in the general population, to ascertain the most likely course of fibroids during and after pregnancy, and to determine what postpartum factors influence regression or growth. We also aimed to understand the risks associated with the presence of fibroids. We have accomplished those goals and published many of our findings with other key papers in the publication pipeline. The first phase of the cohort required as representative a population as possible in order to understand prevalence and determinants of risk for having fibroids. The proposed second phase of the cohort would not face these constraints and can move forward with more detailed investigation across the full range of fibroid characteristics and severity of disease. We recognize by recruiting women who report having been told they have fibroids that we are shifting the sample and believe the net result is helpful to the data. We will now have more participants with fibroids which are likely to be more numerous and larger. That enhances the study's power to address our hypotheses about type, size, and total fibroid volume.

At Women's Health Research at Vanderbilt (Hartmann director), we find that women with fibroids are very interested in participating in research. We believe the shift to include recruitment methods targeted at women planning a pregnancy or in early pregnancy (< 9 completed weeks) will be well received. Given existing bases of operations in three major metropolitan areas (Nashville, Chattanooga, Knoxville), we are confident that we will meet our recruitment goals for the proposed four years of active recruitment and can begin that recruitment immediately. In the interim, while this proposal is under review, we are continuing to conduct first trimester ultrasounds to add to the *Fibroids in Pregnancy Cohort I*, though the formal RO1 funding on this topic is completed. We will also use this interim window to develop and pilot additional recruitment materials. We currently monitor recruitment and ultrasound findings of fibroid status in real time. Should we find in the next phase that we are falling short of targets, we would adapt the protocol to re-instate prior study sites such as those in the Memphis and Raleigh-Durham areas (for which we keep current active IRB). We are confident we can achieve our goals for cohort size and composition.

Potential Questions and Concerns

Is there opportunity for animal research to inform these questions? While Eker rats and some breeds of dogs form nodules of smooth muscle in the uterus, primate implantation, placentation, and parturition are unique. Coupled with differences in reproductive endocrinology and disease course across the life span, we may make the most progress in understanding the risks of fibroids in human pregnancy through careful, population-based observational research and eventual highly selective intervention trials as indicated. If a characteristic of fibroids is found to relate to risk of poor reproductive outcomes, it will help focus basic-translational explorations. For instance, if larger or multiple fibroids are associated with PTB it might direct research towards understanding the influence of fibroids on myometrial contractility, irritability, and prostaglandin pathways, as compared to if sub-placental location is found to be a key to risk. Then it might direct attention to patterns of uterine blood flow, placental aging and infarcts, or coagulation.

What role might differences in defining and measuring fibroids play in why RFTS findings diverge from much of the literature? Operational definitions of fibroids in the literature vary widely. They include self-report of prior diagnosis, history of fibroids detected by imaging prior to the index pregnancy, hospital discharge ICD codes, and referral for fibroids without explaining how actual fibroid status was determined. Use of clinical data is concerning because physicians often fail to consistently record fibroids in medical records or ultrasound reports unless their size or location is believed to be concerning. Anecdotally, omission can be linked to the clinical concept that fibroids are very common, like nevi on skin exams, and not necessarily worthy of note. Physicians also report they do not want to worry women or spend time discussing the implications. As a rule, women with symptoms, a poor reproductive history, more frequent gynecologic care, and those who are easier to examine because of body habitus and/or comfort with exam are more likely to be aware they have fibroids;

and large or submucosal fibroids are more likely to be noted. Smaller intramural fibroids, on the other hand, are ignored or not detected. Even in a best-case scenario, if routine early pregnancy ultrasound was obtained for clinical purposes, detection bias persists: small fibroids are easily missed, may not be noted, and individual sonographers may apply different standards for the lower limit of size they record if documenting fibroids is not an explicit focus of the imaging examination. Women with adverse reproductive outcomes in the past or known fibroids will be more closely evaluated, and fibroids in areas most easily visualized will be more likely to be noted than others, resulting in misclassification of some women with fibroids into the no-fibroids group. The prevalence of fibroids in retrospective pregnancy cohorts, 1-5%,[8, 88] suggests misclassification is at work. To date there are no prospective studies with uniform use of research ultrasound other than RFTS.

Research definitions of fibroids have not kept pace with technologic improvements in ultrasound resolution. Ideally, uniform operational definitions for ultrasound diagnosis and classification of fibroids would be used. (We will be piloting the NIH Uterine Fibroid Classification System led by Dr. James Segars in a sub-project of the proposed work.) The criteria used by Muram and colleagues beginning in the 1980s have provided a consistent standard with some modification for required diameter of the mass. The original criteria were: 1) a spherical mass with a diameter greater than 3 cm; 2) distortion of the adjacent myometrium by the mass; and 3) distinctive echogenicity of the mass differentiating it from the surrounding myometrium.[89] Ultrasound technology has improved dramatically over the intervening decades and smaller masses can be easily visualized with confidence. The majority of reports from clinical data describe routine ultrasound practices, but do not specify the lower limit of size, though we suspect they have fallen with time.[2, 5, 7, 10, 90-93]

Is it plausible that previously reported associations can be explained by confounding? A combination of factors is likely at work: 1) selection and detection bias in study populations; 2) fibroid measurement techniques; 3) data analysis methods; and 4) confounding. Fibroid detection and measurement challenges are noted above. Tertiary care facilities have disproportionately high risk and lower socioeconomic status patients which may influence risk of outcome events. Race and age composition of populations may explain some variation – we have found black women are at increased risk of both SAB and PTB and both age and race/ethnicity are key confounders. A large proportion of clinical publications do not present fully adjusted models. When they do have multivariate models, they lack key candidate confounders such as diabetes status, BMI, smoking, alcohol use, and parity. Failure to use hazard models that accurately take into account time-at-risk-and-in-view introduces bias. Most clinical cohorts for SAB begin after the peak incidence of SAB or draw on rare populations like women having assisted reproductive technology or genetic diagnosis procedures. Hazard models also allow for time-varying covariates and for continuing all members in the cohort until failure or censoring. We find in the *RFTS Fibroids in Pregnancy Cohort I* that the observed effects are indeed the result of confounding and that proper modeling improves both precision and control of confounding.

Wouldn't it be more desirable to launch a separate prospective cohort or to aggregate data from similar studies to confirm the findings of RFTS? It would be ideal to have complete replication of another cohort for the same rationale that we wish to extend this cohort – to determine if the findings are robust and to obtain nuanced information about the potential for differences in effects of fibroids depending on their characteristics. Nonetheless, at present women and their care providers are acting on potentially erroneous information and RFTS is the closest to having precise information. Our cohort is diverse (over-representing black women who are most affected by fibroids compared to national population) and represents seven separate metropolitan areas. The relative value of obtaining this information rapidly is high, compared to deferring to an unknown later opportunity. We cannot identify another team in the United States who has a prospective cohort with whom to aggregate data to achieve the goal of deeper data more rapidly.

Summary

We are confident we can deliver the most informative epidemiologic study to date of the association of fibroids and fibroid characteristics with SAB and PTB. More than one in ten women in the United States has a fibroid during pregnancy and fibroids have been associated with multiple adverse outcomes. At present, the need for removal of particular fibroids or for treatment to stabilize or diminish the size of fibroids before or between pregnancies is unknown. Expanding the *Fibroids in Pregnancy Cohort* to 9,124 women will provide sufficient data to refine understanding of what specific size, type, and locations of fibroids are – or are not – associated with adverse reproductive outcomes. In doing so, we will provide crucial information to gynecologists and prenatal care providers, and women themselves that has value for medical decision making and research.