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Dried blood spots as a tool for measuring ovarian reserve in young female cancer survivors

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ABSTRACT
More than 80% of women who are diagnosed with cancer before the age of 40 will survive long-term. As a result, there are nearly 400,000 reproductive-aged women who have a history of cancer and cancer treatment in the United States. Due to cancer treatment, these young cancer survivors face higher risks for shortened reproductive lifespans, infertility, and primary ovarian insufficiency. Therefore, estimating their remaining ovarian reserve is important to identify potential opportunities for biologic parenthood. Anti-mullerian hormone (AMH) is a glycoprotein produced by early staged ovarian follicles in women and can be used as a sensitive biomarker of ovarian reserve. It has been shown that pre-chemotherapy AMH levels can predict post-chemotherapy ovarian function, making AMH a promising clinical tool for measuring residual ovarian function after cancer. However, logistics and costs of venipuncture blood collection associated with AMH testing have limited our ability to further explore this topic on a large scale in young adult cancer survivors. We have conducted a pilot study in which young adult cancer survivors were asked to collect dried blood spot samples by themselves. My project aims to characterize the feasibility of DBS self collection for measuring AMH in this population.

BACKGROUND
As a result of the advancement in cancer treatment, more than 80% of young girls and women who are diagnosed with cancer before the age of 40 will survive long-term. Consequently, there are nearly 400,000 reproductive-aged women who have a history of cancer and cancer treatment in the United States. It is known that cancer treatments such as chemotherapy, radiation, and endocrine therapy, can have long-term negative effects on a woman’s reproductive health. These late effects of therapy include shortened reproductive lifespans, infertility, and primary ovarian insufficiency that result in a lower overall quality of life. Because most young cancer survivors (YCS) will have some residual ovarian function after cancer treatment, estimating their remaining ovarian reserve important to identify potential opportunities for biologic parenthood.

Ovarian reserve is a term that is used to describe the ability of an ovary to produce a healthy mature follicle for fertilization and conception. Ovarian reserve tests (ORT) are used to help determine a women’s reproductive potential based on the quality and/or quantity of their remaining oocytes. There are several different ORTs that can determine ovarian reserve. Current literature suggests that anti-mullerian hormone (AMH) blood concentrations may be the most sensitive biomarker for ovarian reserve compared to follicle stimulating hormone (FSH), estradiol (E2), antral follicle count (AFC), or Inhibin B.

AMH is a glycoprotein hormone that plays a large role in sexual differentiation in utero as well as ovarian functioning in reproductive-aged women. In adulthood, AMH is exclusively produced by granulosa cells in the ovarian follicles. AMH production is highest in the preantral and small antral stages of follicle development. AMH production stops before the follicle
matures into a tertiary follicle. Because AMH production occurs only in the early stages of follicle development, blood levels of AMH can be used to estimate the size of the primordial oocyte pool in women. AMH levels peak at puberty and begin to decline at age 25, reaching undetectable levels around 40 years old.\textsuperscript{10}

In the context of cancer, AMH levels can help identify ovarian function in young survivors. First, periodic assessment of AMH levels can help physicians identify impact of chemotherapeutic agents on ovarian reserve.\textsuperscript{11} Second, AMH levels can help determine remaining ovarian function after successful completion of cancer treatment.\textsuperscript{12} In addition, pre-chemotherapy AMH levels predict post-chemotherapy ovarian function.\textsuperscript{13} For these reasons, AMH is a promising marker for measuring the residual window of ovarian function after cancer. However, this area of research has been limited by small sample sizes due to the costs and logistics associated with venipuncture blood collection.\textsuperscript{14}

Dried blood spots (DBS) provide a more cost-effective and feasible alternative for AMH testing. DBS is a novel approach that collects drops of whole blood on filter paper following a simple finger prick.\textsuperscript{15} Recent validation work has demonstrated that assays for quantifying AMH in DBS samples are highly sensitive and reliable.\textsuperscript{16} While DBS collections have successfully been performed in large health surveys and/or by trained individuals including healthcare providers and research assistants, self-collected DBSS, has not been described.\textsuperscript{17} If successful, self-collected DBS would facilitate larger sample collections from YCS who are geographically diverse. The objectives of this study is to 1) determine the feasibility of obtaining self-collected DBS to measure ovarian reserve in a large, geographically diverse population of female YCS and 2) to characterize the association of self-collected DBS AMH levels with participant characteristics and exposures.

**DEFINITIONS**

**What are the goals of the project?**
The general goals of this project are to:
- Better understand fertility and ovarian function issues that occur in young female cancer survivors
- Participate in data cleaning, data analysis and manuscript preparation
- Build connections with leading researchers in this area of research

The specific goals of this project are to:
- Characterize the feasibility of self collection for DBS in this population
- Clean primary data
- Learn to use statistical software (STATA or SPSS)
- Analyze primary data: summarize descriptive data, conduct bivariable and multivariable analyses to examine factors associated with DBS-measured AMH levels
- Gain expertise in various AMH assays in order to make appropriate conclusions from lab data

**What is innovative about the project?**
By expanding our knowledge on the implications of using DBS for measuring AMH levels, we are searching for more feasible and cost-effective ways to assess ovarian reserve in a very
vulnerable population (young cancer survivors). AMH blood levels are commonly used in infertility clinics to predict time to pregnancy, patient management with in vitro fertilization, and ovarian reserve. However, many of these patients present with infertility issues unrelated to cancer treatment. It is known that AMH levels and fecundity decrease during chemotherapy and typically continue to stay low 3-6 months after cancer treatment. However, there is a paucity of studies using AMH to define the long-term effects of cancer treatment on ovarian reserve. This project aims to provide an innovative method for measuring AMH. We will test to see if self-collected DBS is a feasible tool for studying ovarian reserve in geographically diverse female young cancer survivors. If so, this may provide a better opportunity at conducting large sample size, longitudinal studies of ovarian reserve in YCS.

**How is the project relevant to a career in medicine?**

Being diagnosed with cancer is very physically and emotionally detrimental. Because of this, I find it particularly important to empower women who have cancer with options that may allow them to have healthy families of their own in the future. As an aspiring OBGYN, this project is especially relevant to my personal career choice in medicine because it focuses on alternative ways to address female infertility. By learning the various etiologies for decreased fertility as well as the methods used to characterize a women’s reproductive capacity, I hope to be one step closer to becoming the kind of physician I wish to be.

**What is the student’s role in and time commitment to the project?**

I will complete the bulk of the data collection, analysis, and write up during the summer of my first year (approximately 40 hours/week from June 29th to Sept 10th). In the first 2 weeks, I will actively engage in literature research to be up-to-date on the research topic, I will take online tutorials to familiarize myself with STATA or SPSS. With my mentor, I will formulate an analysis plan. Then, I will conduct the analysis in parallel with the team’s statistician to learn the skills relevant for this study. By the end of the summer, I will work alongside my immediate supervisors and ISP Chair to write up the results of this study. The paper submission process will most likely extend into MS2 fall quarter.

**METHODS**

**Study population:**

From 2011 to 2013, 295 participants were recruited from the Fertility Information Research Study (FIRST). FIRST is an ongoing prospective cohort study assessing reproductive health in young cancer survivors. Participants were included in the study if they were female, ages 18 to 44, and had a history of cancer or exposure to cancer treatment. This participant pool was invited to participate in a pilot study on using dried blood spots to measure ovarian reserve. Interested survivors were invited to hear about the DBS study in full, after which they provided consent for participation.

**DBS Collection:**

Each consented participant was mailed a study packet that included: an instructional brochure for DBS collection, 2 lancets, a blood spot collection card with 5 DBS circles, various biohazard safety tools, a brief questionnaire on current hormone use and recent menstrual cycle, and a return envelope. Once received, DBS samples were frozen at -80C.
AMH measurements:
AMH will be measured using the picoAMH assay. Ansh Laboratory (Texas) will conduct the assays. DBS standards, samples and controls will be removed from the -80C freezer the day before the assay. Samples will be analyzed for AMH concentration by quantitative, three-step sandwich ELISA immunoassay (Ansh Labs #AL-129). From the DBS collection card, 3.2 mm discs will be punched out and placed in 12X75 culture tubes for elution in extraction buffer. Assays will be run with single spot and corrected for spot dilution.

Statistical analysis

Returned AMH levels will be analyzed using SAS statistical software. Categorical variables will be summarized by frequency and proportion. Continuous variable will be summarized by means, medians, and range. Baseline characteristics of FIRST participants will be compared by DBS study enrollment using Fisher’s Exact or Student’s t-test, as appropriate. AMH levels will be log-transformed to approximate a normal distribution. Analysis of variance will be used to compare AMH levels by participant characteristics. Two-tailed p-values <0.05 will be considered significant.

EVALUATION
I will have accomplished the above goals and successful completed the project if I meet the following criteria:

• Was able to gain the necessary statistical skills to analyze AMH data for the purpose of associating AMH levels with participant characteristics and exposures
• Gained enough knowledge about AMH, DBS, and ovarian reserve in vulnerable populations, like young cancer survivors, to make reasonable clinical suggestions for the utility of AMH testing
• Contribute significantly to the publication of this project
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