COMPARATIVE EVALUATION OF OVARIAN RESERVE FOLLOWING CYTOTOXIC THERAPY FOR BREAST CANCER AMONG WOMEN IN THE REPRODUCTIVE AGE IN SELECT SITES IN NAIROBI KENYA

(A COMPARATIVE CROSS-SECTIONAL STUDY)

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H58/45888/2016
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A Research Proposal Submitted in Partial Fulfillment for the Degree of Masters of Medicine in Obstetrics and Gynecology,

University of Nairobi.

2021

DECLARATION

This research was undertaken in part fulfillment of the Masters of Medicine in Obstetrics and Gynecology from the University of Nairobi and was be my original work and has not been undertaken and presented for a degree in any other University.

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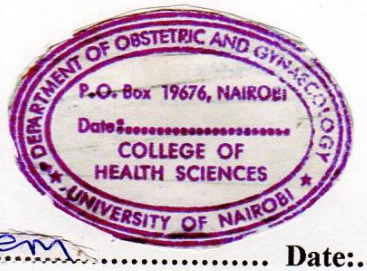

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FUNDING AGENCY

The study was funded by the Kenyatta National Hospital research and Programmes department, upon approval by the KNH/UON ethics research committee

ACKNOWLEDGEMENTS

I wish to acknowledge and thank the Lord Almighty for the good health as I pursued this course. Special thanks to my supervisors, Professors SBO Ojwang' and Christine Kigondu and Doctors George Ogweno and Catherine Nyongesa for their dedication to the development and review of the study subject; to the research assistants, Lucas, Solomon and Anne coordinating the identification of follow up of the participants; to the Clinic 66 Nurses; to the team at the four study sites, Kenyatta National Hospital, Texas Cancer Center, Faraja Cancer Center and Nairobi Radiotherapy and Cancer Center; to the patients, both breast cancer survivors and non-cancer patients in clinic 66; and finally to my family for their prayers and understanding during the time that I was away in school

DEDICATION

The dissertation is dedicated to my wife, Lilian Nandili, our Children, John Chrysostom, Megan Hadassa and Jedidia Beato and the all the Cancer Survivors

LIST OF ABBREVIATIONS

AF	-	Antral Follicles
AFC	-	Antral Follicle Count
AMH	-	Anti Mullerian Hormone
BOV	-	Basal Ovarian Volume
CCCT	-	Clomiphene Citrate Challenge Test
CME	-	Continuous Medical Education
CMF	-	Cyclophosphomide, Methotrexate, Florouracil
CRA	-	Chemotherapy Related Amenorrhea
CV	-	Co-efficiency of Variation
E2	-	Estradiol
EFFORT	-	Exogenous Follicle Stimulating Hormone for Ovarian Reserve Testing
ERC	-	Ethics Research Committee
FAC	-	Fluoro-uracil, Anthracycline, Cyclophosphamide
FSH	-	Follicle Stimulating Hormone
GI – PGF	-	Gonadol Insufficiency – Premature Gonadol Failure Syndrome
GnRH	-	Gonadotropin Releasing Hormone
GAST	-	Gonadotropin Releasing Hormone Agonist Test

HPO	-	Hypothalamo – Pituitary – Ovarian axis
HRT	-	Hormonal Replacement Therapy
IVF	-	In Vitro Fertilization
KNH	-	Kenyatta National Hospital
ORT	-	Ovarian Reserve Test
OV	-	Ovarian Volume
PCOS	-	Polycystic Ovary Syndrome
PI	-	Principal Investigator
SOP	-	Standard Operating Procedure
TGFb	-	Transforming Growth Factor b
TVU	-	Trans Vaginal Ultrasonography
UON	-	University of Nairobi

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

ABSTRACT

Introduction: While advances in breast cancer therapies have led to improvements in long-term survival, treatments often lead to premature ovarian failure and sometimes infertility. This is dependent on the age, dose and type of the agent. In evaluating the extent of this damage, Ovarian Reserve Testing (ORT) has become established as a pre and post chemotherapy fertility counselling tool and as a guide to the various treatment options. Despite paucity of data from developing countries, literature from the developed countries indicate promising results with the use of Anti Mullerian Hormone (AMH) to ovarian reserve. In addition to being relatively stable across the menstrual period, AMH appears to show significant comparable estimates of ovarian reserve across different populations.

Objective: To evaluate the impact of cytotoxic therapy on ovarian reserve in patients in the reproductive age at least one year after chemotherapy for breast cancer at a select number of cancer treatment centres in Nairobi

Methodology: This was a comparative cross-sectional study. Following Ethical approval from the KNH-UoN ERC, a total of 40 women attending breast cancer clinics and meeting the inclusion criteria were selected from three cancer treatment centres in Nairobi (Kenyatta National Hospital, 22; The Texas Cancer Centre, 12 and the Nairobi Radiotherapy and Cancer Centre, 6). A comparative group of 40 women, matched for age (+/1 3years) were identified from the 'well woman clinic 66' at the Kenyatta National Hospital Clinic. Data was collected using an interviewer guided questionnaire administered to the participants; clinical data was abstracted from the patients' files. Two ml of venous blood was drawn by principal investigator or trained research assistant and taken to the Lancet laboratory for evaluation of AMH levels. Data was cleaned and analyzed using SPSS Version 23 software.

Results: The test of normalcy was carried out for the 80 participants. The mean age and the BMI for the chemotherapy group was higher than that of the comparative group (39.23 vs 33.90, f=0.176, p=0.672; 30.74 and 28.34, f=0.002, p 0.964; 14.28 vs 13.90, p, 0.230 respectively). The most common histological diagnosis for breast cancer was invasive ductal carcinoma (29, 83%), while the most commonly used chemotherapy treatment was a combination of Adriamycin, cyclophosphamide and paclitaxel (16, 40%). All the 40 cancer participants exposed to chemotherapy had lower than normal levels of AMH; 12 (30%) of those in the comparison group had lower than normal levels (less than 0.15ng/ml). There was a negative correlation between the AMH levels and the patients age and the period of resumption in menses (pearsons test, -0.369, p 0.047 and 13.24, p, 0.141 respectively). A moderately strong positive correlation exists between AMH levels and the time since the last chemotherapy. As a secondary objective, most (90%) of the health care workers did not discuss fertility related implications of cancer treatment with their patients.

Conclusion and Recommendations: It is imperative that a discussion about treatment options for cancer and fertility is held by the clinicians taking care of young women with cancer. The use of AMH to assess ovarian function pre and post treatment should be more widely made available and a consideration on more oophoro protective regimens considered. Lastly, institutions managing young women with cancer should consider implementing the fertility assessment protocols to be used in the course of offering this treatment.

Key words: Anti Mullerian Hormone, Cancer, Chemotherapy, Ovarian Reserve Volume

1.0 INTRODUCTION

1.1 Background

Globally, an estimated 10–20% of newly diagnosed cancers occur in women of childbearing age (1). In addition, childhood cancer treatment has improved dramatically resulting in current overall survival rates of over 85 to 90%(2). Although breast cancer has typically been a disease affecting women in their 5^{th} decade, approximately 30% of cases are being diagnosed in women less than 40 years of age(3).

As a result of the improvements in early detection and adjuvant chemotherapy for breast cancer, there is notable increase in incidence and improvement in survival(4). Comparative studies have shown that younger women with breast cancer are at a higher risk of disease progression(5), and are likely to receive adjuvant chemotherapy, which has been shown to be more beneficial(4).

As previously reported, chemotherapy induces premature ovarian failure in 20%–80% of premenopausal women(7), with the wide range reflecting variations in age at treatment, follow-up duration, the definition of ovarian failure, and the chemotherapy regimen studied. Consequently, long-term toxicities of breast cancer treatment, including gonadal damage and infertility, are of increasing relevance especially among young breast cancer survivors(6).

As reported by Partridge et al., fertility after treatment was a concern for approximately 60% of young women present with early breast cancer and that fertility concerns would influence the treatment decisions of 26% of these women(8). This study was therefore conceptualized with the

aim of evaluating ovarian reserve using AMH, among women in the reproductive age group who have undergone chemotherapy treatment for breast cancer.

1.2 The Ovarian Function

Although follicle formation and folliculogenesis is well documented for many mammalian species, there is poor understanding of the paracrine and autocrine control of follicular reserves and entry of follicles into the growth path towards atresia or ovulation(11). As shown in figure 1, several models have been described to accurately chart the progressive development of the follicles, the effect of the interplay of the paracrine and the autocrine factors, with subsequent ovulation, atresia and decline of primordial follicles in the ovary with age(12).

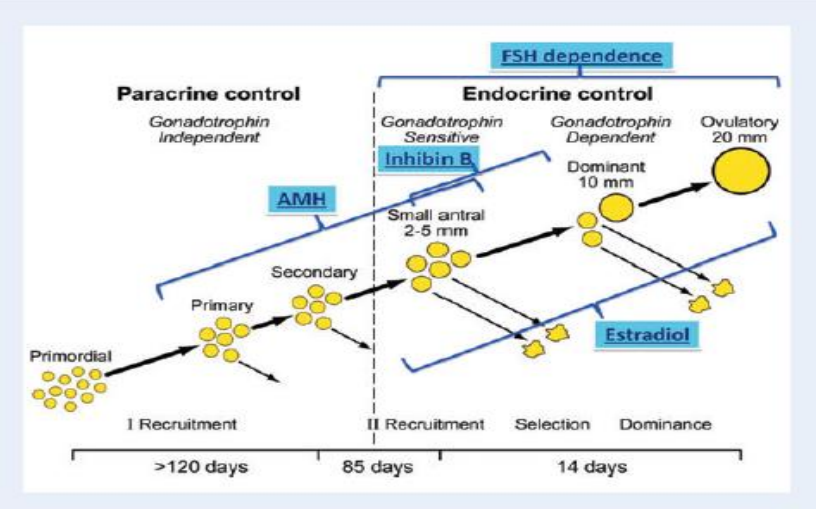

Figure 1: *Schematic Representation of Follicle Development, showing AMH production in Early Stages of Follicle Development(12)*

1.3 Ovarian Damage following Chemotherapy

Although the gonadotoxic effect of chemotherapeutic agents is well documented, the prevailing mechanisms are not fully understood(13). It is however postulated that both oocytes and ovarian follicles may be affected by chemotherapy(2). The extent of damage from the chemotherapeutic

agents is influenced by age at time of treatment, percentage of resting oocytes, and the type, dose, and schedule of chemotherapy; in addition, there is a cumulative, dose-dependent relationship between the drug and gonadal toxicity(10). Table 1 shows the classification of chemotherapeutic agents based on the different levels of the potential damage caused to the ovarian tissue(14).

Table 1: *Class of Chemotherapy, their Action and Risk of Infertility (14)*

Class of agents	Examples	Mechanism of action	Infertility risk
Alkylating agents	Cyclosphomide Mechlorethamine Chlorambucil Melphalan	The active metabolites form cross - links with DNA with resultant inhibition of DNA synthesis and function. DNA double strand breaks and resultant P63-mediated apoptosis in human primordial follicles	High risk
Platinum based compounds	Cisplatin Carboplatin	Covalently binds to DNA to form intra and inter strand DNA cross – links leading to DNA breakage during replication. This inhibits DNA transcription, synthesis and function. Specific toxicity has not been shown in human primordial follicles	Intermediate risk
Antimetabolites	Methotrexate 5 – fluorouracil Cytarabine	Inhibition of DNA, RNA, thymidylate and purine synthesis. No DNA damage in human follicles hence not gonadotoxic	Low risk
Vinca alkaloids	Vincristine Vinblastine	Inhibition of tubulin polymerization and disruption of microtubule assembly during mitosis. This arrests mitosis during metaphase and leads to cell death. No DNA damage in human follicles, hence not gonadotoxic	Low risk
Anthracyclin antibiotics	Daunorubicin Bleomycin Adriamycin (Doxorubicin)	Inhibition of DNA synthesis and function. It interferes with DNA transcription. Inhibits topoisomerase II, leading to the DNA breaks. Also forms toxic oxygen free radicals which induce DNA strand breaks, thereby inhibiting DNA synthesis and function. Doxorubicin induces DNA double strands	Low risk (except Adriamycin: intermediate risk)

1.4: Chemotherapy in Patients with Breast Cancer

According to the European Society of Medical Oncology (ESMO) guidelines on the management of breast cancer, the decision on systemic adjuvant treatment is based on: the predicted sensitivity to particular treatment types; the benefit from their use and an individual's risk of relapse (15).

The predicted treatment sequelae, the patient's biological age, general health status, comorbidities and preferences are also important considerations (15).

Chemotherapy is recommended in the vast majority of triple-negative, HER2-positive breast cancers and for high-risk luminal HER2-negative tumours (15). The benefit of using chemotherapy is more pronounced in ER-negative tumours compared to ER positive tumours in which chemotherapy partially exerts its effect by induction of ovarian failure (15). With the development of more effective and less gonadotoxic drugs, the preferred chemotherapy regimens have changed over time, with greater use of anthracyclines and taxanes (which have a more variable effect on ovarian function) and with the introduction of trastuzumab (for which no published data about ovarian toxicity are available) (2).

Additionally, the role of GnRH agonists in preventing chemotherapy-related ovarian failure was reported and supported by the efficacy data (less premature ovarian failures and more pregnancies) from the POEMS trial (ER-negative patients) and safety data from TEXT trial (ER-positive patients) (15). However, due to contradictory results, the decision must be taken in a case-by-case manner and after careful discussion with the patient regarding the benefits and risks (15).

1.5: Signs and Symptoms of Ovarian Failure

The signs and symptoms of Premature Ovarian Failure (POF) include vasomotor symptoms such as hot flushes and night sweats, genitourinary symptoms including vaginitis, dyspareunia and dysuria and osteoporosis(16). Shapiro et al further demonstrated that POF after adjuvant chemotherapy for breast cancer is associated with rapid bone loss in early stage breast cancer(17).

Consequently, patients who have undergone chemotherapy may present with chemotherapy-related amenorrhea (CRA)(18) as a result of the toxic effect of the chemotherapy on ovarian tissue(19). As shown in table 2, different classes of drugs cause variable levels of ovarian damage as measured by the incidence of amenorrhea (20).

Table 2: *Gonadotrophic Effect of Breast Cancer Chemotherapy Regimens*

Author	Adjuvant chemotherapy	Incidence of Amenorrhea
Goldhirsch et al	Classic CMF	61% (<40yrs): 95% (>40yrs)
Bines et al	AC	34%
Nabholtz	FAC	32.8%
	TAC	51.4%
Hortobagyl et al	Doxorubicin – based	59%
Levine et al	CEF	51%

CMF = Cyclophosphamide, Methotrexate, 5 Flouro – Uracil: AC = Doxorubicin, Cyclophosphamide
FAC = 5 Flouro – Uracil, Doxorubicin, Cyclophosphamide: TAC = Docetaxel, Doxorubicin, Cyclophosphamide: CEF = Cyclophosphamide, Epirubicin, 5 Flouro – Uracil

N.B: Impact of taxanes and endocrine treatment with tamoxifen or aromatase-inhibitors on the incidence of chemotherapy-induced amenorrhea is unclear (20)

Though influenced by age and duration of follow up, restoration of menstruation after CRA is possible (14); this is estimated at 39–55% in younger women (less than 40 years) and 0–11% in older patients (more than 40 years) (18). Women who maintain normal menses throughout chemotherapy however still remain at risk for developing POF(21).

2.0 LITERATURE REVIEW

2.1 Introduction

Estimation of functional ovarian reserve in patients can have a significant impact on how patients are counseled both before and after chemotherapy and radiotherapy and subsequently on their quality of life (14). Information about ovarian reserve can be used to predict the response to ovarian stimulation, hence discuss future fertility preservation practices (14) such as embryos cryo preservation, In Vitro Fertilization (IVF), or undergo counseling regarding adequate contraception.

2.2 Classification of Ovarian Reserve Tests

Ovarian reserve testing (ORT) is done to assess the reproductive potential of a given individual(2). The tests have the potential to estimate the reproductive lifespan of the ovaries, which would allow an accurate estimation of fertility status and the risk of premature ovarian failure (POF) (22). As shown in figure 2, various ORTs have a good predictive value for ovarian function(23).

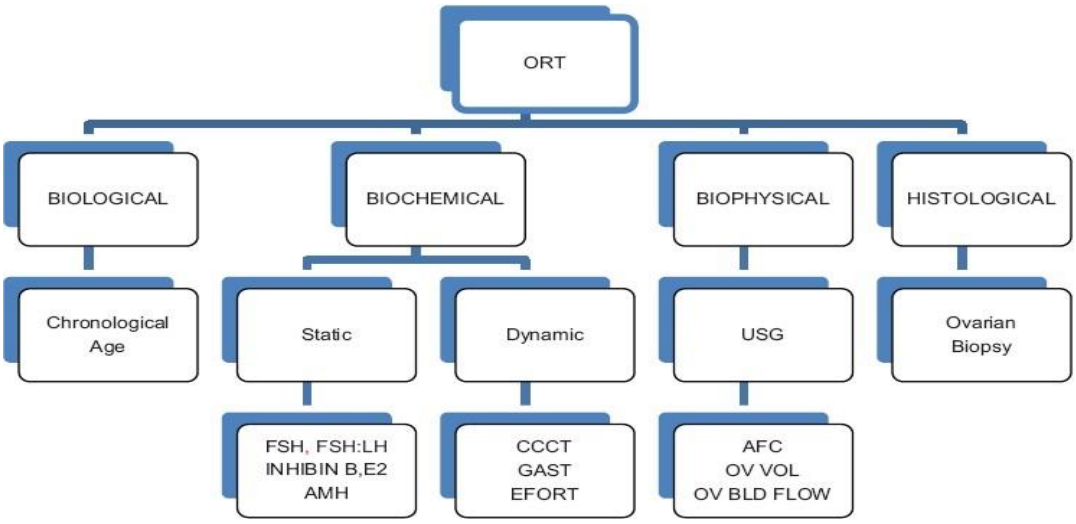

Figure 2: *Types of ovarian reserve tests(24)*

The interpretation of the result, however, is complicated by the lack of uniform definitions for poor or hyper-responders and uniform threshold values to identify abnormal results (24); proposals have

been advanced about the use of multiple markers to improve on the predictive value of the tests(25). An ideal ORT, therefore, is easy to perform, reproducible, and the decisions based on their results are able to help differentiate women with a normal and poor ovarian response (24).

2.2.1 Biological Markers

2.2.1.1 Chronological Age

Ovarian aging or failure potentially predicts age at menopause, a measure which is known to be highly variable (26). Even though fertility does not decline uniformly in women, age is known to be the most important factor determining the pregnancy potential in regularly cycling women (30). Chronological age alone however has a limited value in predicting individual ovarian responses (31). The age dependent loss of fertility in females (beginning in the 20s and more abrupt in late 30s) is dictated by a continual process of follicle depletion leading to a reduction in both oocyte quantity and quality (27) as shown in figure 3.

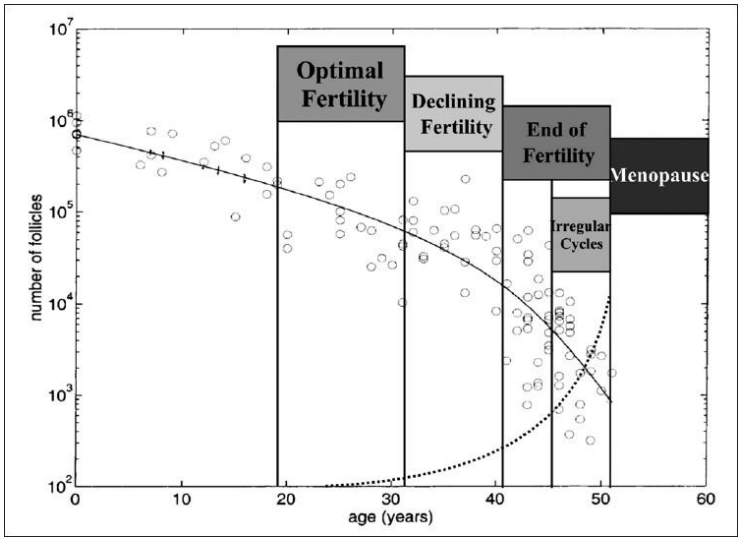

Figure 3: *Quantitative (solid line) and qualitative (dotted line) decline of the ovarian follicle pool(27)*

2.2.2 Biochemical markers

Though with considerable variability, the following biochemical markers have been used to assess ovarian function.

2.2.2.1 Anti Mullerian Hormone

Anti-Mullerian Hormone (AMH), a dimeric glycoprotein, is produced by granulosa cells of preantral and small antral follicles (AFs) (24). The production of AMH follows follicular transition from the primordial to the primary stage, and it continues until the follicles reach the antral stages, with diameters of 2-6mm(32).

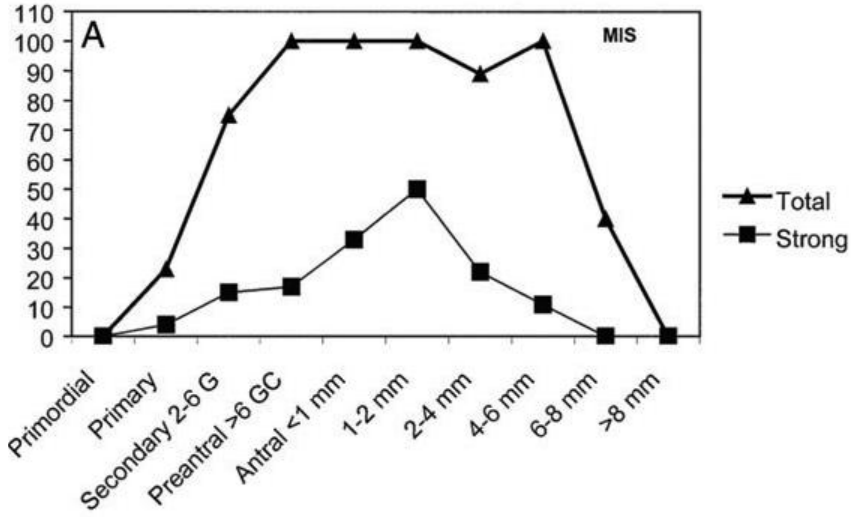

Figure 4: *AMH concentrations across the size of maturing follicles(32)*

It is evident that AMH is the best currently available test in terms of sensitivity and specificity as opposed to, FSH, E2 and inhibin B concentrations or various ovarian challenge tests (33). The levels of AMH strongly correlate with basal antral follicle count (AFC) measured by trans vaginal ultrasonography (TVU)(34). Additionally, AMH can be measured in blood on any day of the cycle (35) with minimal inter cycle variability(36). Threshold values ranging between 0.2–1.26 ng/ml, have been used to identify poor responders with 80–87% sensitivity and 64–93% specificity(25).

Age is an independent influencer on AMH levels. A clear decline in AMH levels with advancing age is demonstrated from various longitudinal studies(37). Freeman et al. in their study, demonstrated that AMH strongly predicted time to menopause(24). To improve on the accuracy

of its interpretation, the use of nomograms identifies the age-related physiological decline in the AMH levels(38). Collectively these nomograms show that AMH levels are low during pre-pubertal development, rise during early puberty and reach a plateau 20–25 years of age, followed by a gradual decline thereafter until becoming undetectable around menopause (Figure 5) (39).

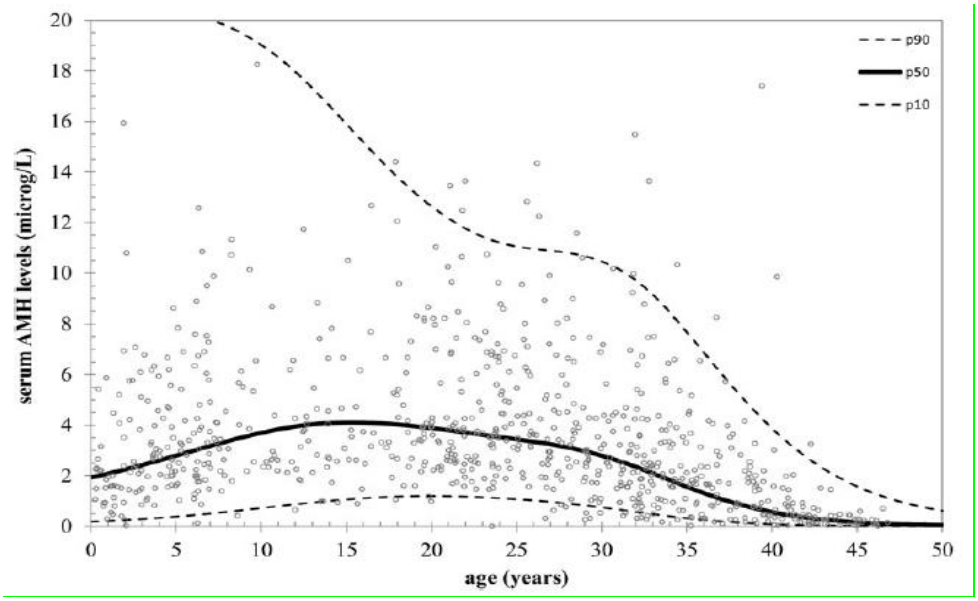

Figure 5: *Nomogram for Anti-Mullerian Hormone Levels(40)*

2.2.2.1.1: Anti-Mullerian Hormone Assay

Assays developed by Diagnostic Systems Laboratory (DSL) and by Immunotech (IOT), applying different AMH antibodies have been used in human female studies (10). Following a merger of the two companies, a new two-step, sandwich-type enzymatic, microplate assay (the AMH gen II assay) was introduced. With this new test, a more stable antibody is used to bind to the mature region of AMH along with the IOT calibrator standard curve and AMH levels can be measured in 20 ml serum in, 3 h(43).

AMH assays also show inter and intra-assay variation (5%) where measurements were performed in the same laboratory(44). Inter laboratory variabilities may be due to dissimilarities in storage

and shipping conditions or differences in the work-up of the manual enzyme-linked immune-sorbent assay (ELISA) test system(45). For samples stored at room temperature for 7 days the AMH serum levels increase; samples stored at -20^{o} C yielded on average 23% higher values, while the same samples stored at -80^{o} C showed no change(44).

Sample instability may also be the effects of complement binding leading to test results that are lower than expected(44). This observation is highest in freshly drawn samples and can be avoided, or minimized, by adding a buffer to potentiate AMH stability at all temperatures or by automatic pipetting or centrifugation of samples within 5 hours of sample collection(44).

2.2.2.1.2: Factors Influencing AMH levels

In addition to the laboratory related variations in AMH levels, other factors influence the levels of AMH. Some studies show these to be limited (37) and merely represent fluctuations by chance, possibly resulting from the gradual changes in the number of antral follicles present in both ovaries (46). However, other studies have demonstrated substantial fluctuations in the menstrual cycle (37), which would argue in favor of measuring AMH levels at the early follicular phase only. This variation occurs more commonly in young women, and therefore needs to be taken into consideration during the assessment(37).

In a cohort study in 2000 women, it was demonstrated that AMH levels decrease under current use of oral contraceptives (47) although previous use of oral contraception did not demonstrate this association; AMH levels increased after discontinuation of oral contraceptives (37).

Studies have also demonstrated that concurrent use GnRH agonists is associated with significantly lower AMH levels(2), making AMH measurement un reliable among such patients. Other factors that seem to influence absolute AMH concentrations, include overweight(24), ethnicity(48), Vitamin D status(49), polymorphisms of AMH and its receptor and genetic variants across the genome (50). Current smoking is associated with lower AMH levels (47).

2.2.2 Other Markers of Ovarian Function

Basal serum **Follicle Stimulating Hormone** (FSH) is one of the longest established parameters for estimating ovarian reserve, predicting ovarian response and pregnancy outcome in IVF cycles (31,51). Several studies have concluded that age better reflects oocyte quality whereas FSH reflects oocyte quantity(31). Combined with the relative practicality, patient tolerability and low cost of performing the test, bFSH remains one of the most commonly performed tests of ovarian reserve(14).

Increasing **Estradiol** concentrations on day 3 of the cycle are associated with decreasing oocyte numbers and pregnancy rates(59). In a study conducted by David Seifer, poor ovarian response was more commonly seen in those with <20 or >80 pg/ml of estradiol but did not show any correlation to the pregnancy rate (24).

Serum dimeric inhibin B: There is evidence that early follicular inhibin B levels correlate with follicle cohort size(64). Therefore, inhibin B levels on cycle day 5 have been shown to be predictive as an early indicator of response during ovarian stimulation as well as outcome(65). **Inhibin A** has been used in combination with the clomiphene citrate challenge test (CCCT)(67)

GnRH agonist test (GAST) (68) and the exogenous FSH stimulation test (EFORT) (10) to predict ovarian responsiveness.

Clomiphene Citrate Challenge Test (CCCT) involves the administration of 100 mg of clomiphene citrate from the fifth day of the cycle for 5 days(27), with subsequent assessment of bFSH on day 3 of the cycle and stimulated FSH levels on day 10. In addition, it has the drawback shared by all dynamic tests in that it is expensive, more invasive, more time consuming, and associated with the possible side effects of administered drugs(70).

Antral Follicle Count (AFCs) are measured by trans-vaginal ultrasonography (TVU) in the early follicular phase, by taking the mean of two perpendicular measurements. The numbers of follicles in both ovaries are added for the total AFC. AFC has long been used as a marker of ovarian reserve (73), with a count of 8–10 is considered as a predictor of a normal response as shown in figure 6.

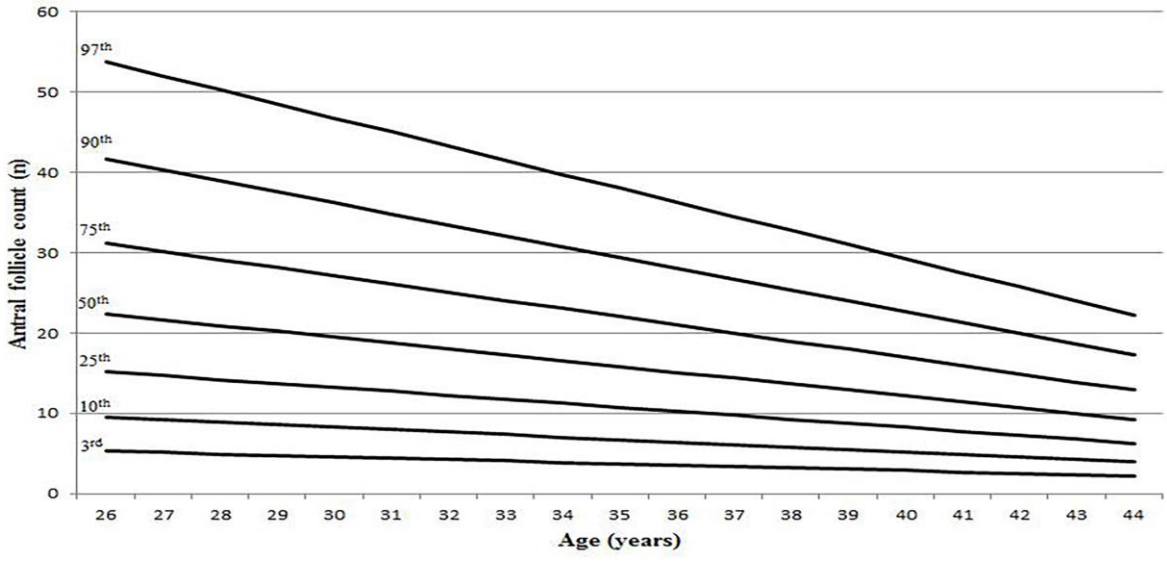

Figure 6 *: Nomogram for the Antral Follicle Count Reduction with Age (40)*

Ovarian Biopsy done at laparoscopy or laparotomy has shown that the follicular density reduces with age and is correlated with the ovarian volume in women >35 years of age (80). Also, women with unexplained infertility have fewer follicles than those with tubal factor infertility (80). It is understood that an invasive ovarian biopsy does not add to the information available through noninvasive modalities and it is not recommended to be used as an ORT (82).

2.4: Application of Ovarian Reserve Testing

Different areas of reproductive medicine exist where ovarian reserve testing may prove to be of distinct clinical benefit as follows:

2.4.1: Chemotherapy and Radiotherapy

Following adjuvant chemotherapy for cancers, ovarian failure commonly occurs in women, with age being an independent influencer on the outcome. Hormone profiles obtained from these women correlate with post-menopausal levels, with low levels of estradiol and progesterone, coupled with elevated FSH and LH levels (2).

During chemotherapy, AMH levels drop with some recovery 3–6 months thereafter. AMH, both before and after treatment, may be useful in the management of young women diagnosed with cancer, since many women are concerned about their future fertility potential(83) and fertility preservation may be considered. Measuring AMH levels in young women surviving cancer would also help to forecast the long-term reproductive outcomes(2).

2.4.2: Other Areas of Relevance for Anti-Mullerian Hormone Assessment

Fecundity and Menopause Prediction in the General Population, largely as a result of the ongoing trend of delayed childbearing especially in the western countries, with age related reduced probability of spontaneous pregnancy(2). Steiner et al documented a good correlation between initial AMH concentrations and natural fertility among women 3 – 34 years (84).

Patient Management in In Vitro Fertilization: Most published studies about AMH and subsequent IVF outcomes have been carried out in heterogeneous patient cohorts, with conflicting results with regard to the capacity of AMH to predict treatment outcome(8). However, a relationship between the number of oocytes retrieved and pregnancy exists, with a response of between 9–13 or 6–15 oocytes associated with the highest pregnancy or live birth rate (47).

3.0 CONCEPTUAL FRAMEWORK

Ovarian function and fertility preservation in females undergoing treatment for cancer depend on the patients' age, type of cancer, type of chemotherapy, patient's BMI, other medical conditions such as PCOS and other habits such as smoking. Among the various tests used to assess ovarian reserve, data suggests that AMH and AFC are the most sensitive measures of diminished ovarian reserve among female cancer survivors (8) as their levels reflect the number of preantral follicles. Levels of AMH also fluctuate minimally during the menstrual cycle (27, 28). Previous reports indicate that AMH levels decline with age, predict time to menopause, predict pregnancy after in vitro fertilization, and are associated with fecundity in the general population (10). Figure 7 depicts the interplay of the various factors and the influence on ovarian reserve.

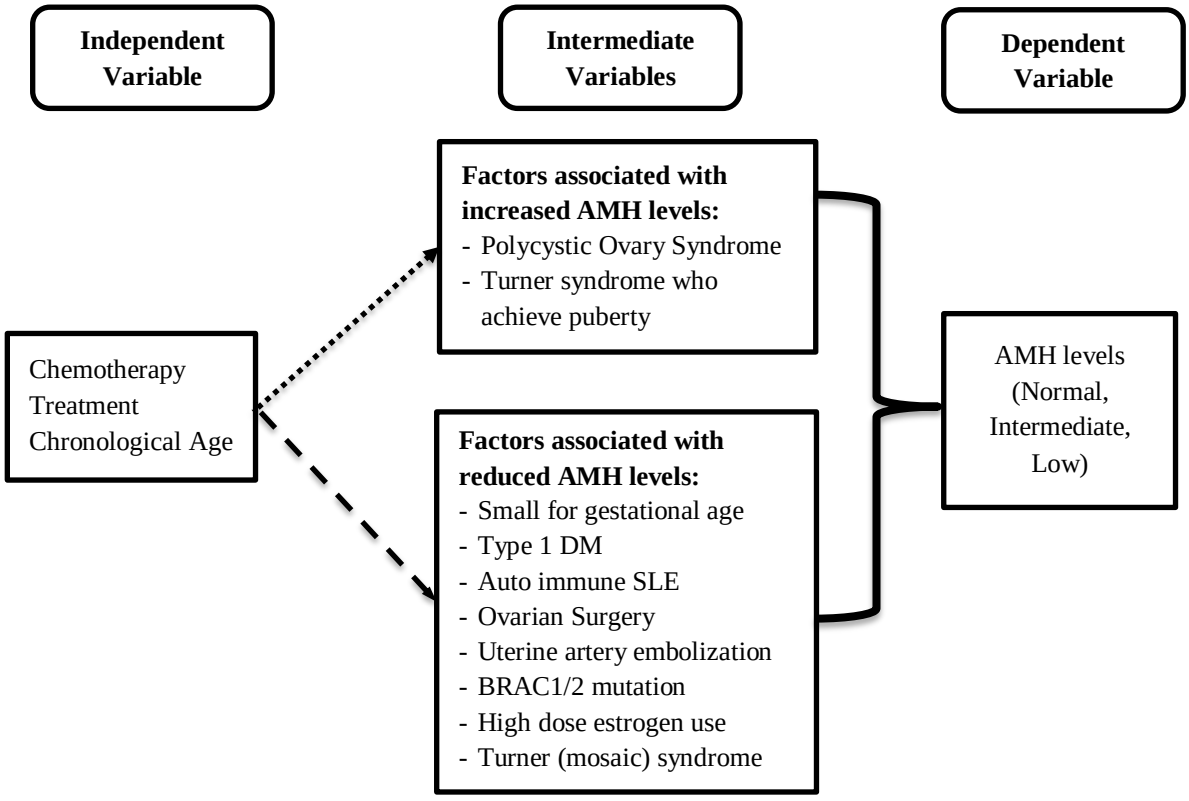

Figure 7: *Conceptual Framework*

4.0 STUDY JUSTIFICATION

Due to the effect of chemotherapy on fast regenerating tissues such as the ovary, patients undergoing chemotherapy are at high risks of developing chemotherapy - induced premature ovarian failure, which currently can easily be identified using tests such as AMH or AFC. For these patients who originally had expectations of a normal reproductive lifespan, the realization that they might suffer reduced fertility and a premature menopause can have a profound impact on their self-esteem and quality of life.

Data on female fertility during and after any type of chemotherapy in the developing countries is limited despite more and more women in the reproductive age group being affected by breast cancer. Studies done in the developed world demonstrate a significant decline in serum AMH

levels after chemotherapy for breast cancer, with a majority of the studies being purely descriptive in nature.

Measurement of ovarian reserve in cancer survivors may therefore increase understanding of a woman's reproductive potential after cancer chemotherapy. In addition, accurate identification of patients who are at risk for infertility or poor response to fertility treatments can help physicians to individualize counselling, and can help patients to understand their chances of achieving a pregnancy.

In this study, AMH assessment, which has been shown to be a more superior measure of OR, was used to assess POF and associated factors among female breast cancer survivors in the reproductive age group. Secondarily, an assessment of fertility screening by health care workers aimed at developing an effective surveillance protocol for early detection of compromised ovarian function was done.

4.0 RESEARCH QUESTION

Using Anti Mullerian Hormone, what is the impact of chemotherapy on ovarian reserve in female patients in the reproductive age group, at least one year after treatment for breast cancer in Nairobi Kenya?

5.0 STUDY OBJECTIVES

5.1 Broad Objective

Using Anti Mullerian Hormone, assess the impact of chemotherapy on ovarian reserve in patients in the reproductive age group at least one year after treatment for breast cancer in Nairobi Kenya

5.2 Specific Objectives

Among women in the reproductive age group:

1. To describe the socio-economic characteristics across cancer survivors in comparison to women attending the 'well woman' clinic and without a diagnosis of cancer
2. To determine the levels of Anti Mullerian Hormone as a measure of ovarian reserve in patients treated with chemotherapy in comparison to women attending the 'well woman' clinic and without a diagnosis of cancer
3. To correlate the socio demographic and clinical characteristics with Anti Mullerian Hormone levels at least one year after chemotherapy

5.3 Secondary Objective

Assess the fertility screening practices by health care workers before and after administration of chemotherapy among patients with breast cancer

6.0 METHODOLOGY

6.1: Study Design

The study adopted a Comparative Cross-Sectional study design.

6.2: Study Site and Setting

The study participants on follow up for breast cancer treatment were drawn from the Kenyatta National Hospital (KNH), the Texas Cancer Center (TCC) and the Nairobi Radiotherapy and Cancer Center (NRCC). For the comparative group, the participants were drawn from the 'well woman' clinic 66 at the KNH.

The **KNH** is a public teaching and referral hospital serving patients from a broad socio – cultural divide not only in Kenya but in East and Central Africa. The oncology unit is managed by a multi-disciplinary team of professionals from different departments including oncologists, nurses trained in oncology, nutritionists, social workers, laboratory, pharmacy, radiology and hematology. On average, **200** patients are reviewed in the oncology clinic in a month. Breast cancer remains the commonest type of cancer at the KNH Cancer Treatment Center (CTC) with an average of 100 patients managed for breast cancer per month. The clinic at the CTC operates twice per week, on Wednesdays and Thursdays. On average, 28% of the patients attending the breast cancer clinics are aged between 25 and 40years (89).

The **TCC** and the **NRCC** are privately owned oncology centers in Nairobi. It serves patients from across the country and across the socio – economic divide. It has a bed capacity of **60** with an average of **120** patients attending the hospital for the management of breast cancer per month. The **NRCC** was predominantly set up as a breast cancer treatment center that over time had expanded to cover other cancers. Despite not having an in-patient unit, it serves an average of **80** patients with breast cancer per month. Among these cancer survivors, 30% are between the ages of 25 to 40 years. The three cancer treatment centers have adopted the ESMO guidelines for the management of patients with breast cancer (15).

The KNH 'well woman' **clinic 66**, caters for the reproductive health needs of women, including cervical cancer screening, contraception and gynecological urology reviews. For the year 2017, a total of **950** women were reviewed at this clinic, with the following services: Contraception (52%), cervical cancer screening (20%) and treatment and management of obstetric fistulae (38%). Out

of the women provided with Contraceptive services, 80% used hormonal while 20% used non hormonal methods. Of those who used the non-hormonal methods of contraception, 90% were aged between 25 to 39 years.

6.3: Study Population

Female cancer survivors aged between 25 and 45 years, and who had received adjuvant chemotherapy at least one year at the time of data collection and on follow up at the KNH, TCC and NRCC were selected to participate in the study. The comparative group were selected from females matched by age, and attending the gynecology clinic 66 at the KNH and without a prior history of chemotherapy or diagnosed cancer.

Table 3: *Study Enrollment Criteria for the Study Group (cancer survivors)*

Inclusion Criteria	Exclusion Criteria
• Women aged between 25 and 39 years • Histologically confirmed diagnosis of breast cancer • At least one year after intent to treat chemotherapy and without evidence of recurrence • Presence of the uterus and ovaries	• History of a brain or ovarian cancer • Pregnancy or lactation within three months • Hormonal contraception or hormonal replacement therapy within 4 weeks • History of pelvic irradiation, ovarian surgery, investigation for infertility

Table 4: *Study Enrollment Criteria for the Comparison Group (Healthy Volunteers)*

Inclusion Criteria	Exclusion Criteria
• Women aged between 25 and 39 years • Presence of uterus and both ovaries • Receiving services other than hormonal contraception	• History of a brain or gynecological cancer • Pregnancy or lactation within three months • Hormonal contraception or hormonal replacement therapy within 4 weeks • Any other medical condition other than cancer, associated with ovarian dysfunction • History of pelvic irradiation, ovarian surgery

6.4: Sample Size and Sampling Procedures

The sample size was calculated using the formula for comparing means:

$$n_1 = \frac{(r+1)}{r} \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{\text{difference}^2}$$

Based on a similar study conducted by Anne Partridge et al (8), where there was a statistically significant difference between the AMH levels of female cancer survivors in comparison with the controls (mean, survivors 0.6ng/ml, control 1.8ng/ml, p = 0.0004), the following assumptions were used in calculating the sample size:

n_1	= Size of survivors group	
r	= ratio of exposed group to non exposed group	= 1
σ	= standard deviation of the characteristic	= 1.9
difference	= clinically meaningful difference in means of the outcome: 1.8 – 0.6 = 1.2	
z_β	= corresponds to the power of the study	= 80%
$n_{\alpha/2}$	= corresponds to two – tailed significance level	= 1.96 for α = 0.05

Substituting the above values into the equation gives the sample size n_1

$$= \frac{(1+2)}{2} \frac{1.9^2(0.84 + 1.96)^2}{1.2^2}$$

$$= (1.5)\ (28.8) = 40$$

Therefore, a total of 80 participants: 40 cancer survivors and 43 patients from the well woman clinic were enrolled into the study.

6.5: Sampling and Enrollment Procedure

For the 40 participants in the cancer survivors' group, proportionate sampling was be applied to arrive at the number sampled from each of the hospitals based on the patient numbers as shown in the table below:

Table 5: *Proportions of Patients from the Three Cancer Treatment Centers*

Cancer Treatment Center	**Number Sampled**
Kenyatta National Hospital	22
Texas Cancer Center	12
Nairobi Radiotherapy and Cancer Center	06
Totals	**40**

Sequential sampling was used to identify all the 80 participants for enrollment into the study. For either of the study groups, health talks were held by the Principal Investigator (PI) or Research Assistant (RA) at the respective sites to sensitize the patients and health care workers about the study. Patients aged between 25 and 45 years old, and who completed their chemotherapy treatment at least 12 months prior to the date of data collection were identified from the registry. The patients' files were then marked with a red sticker for ease of identification on the day when they attended their routine clinics. On average, two participants were enrolled per clinic day until the targeted sample of 80 was arrived at.

Upon verbally accepting to participate in the study following the health talk, all potential study participants were escorted to a private room within the clinic. From here, the written consent, either in English or Swahili, was administered by either the PI or RA. Those who declined to further participate in the study were excluded.

6.8: Study Procedures

6.8.1 Blood Collection and Processing Procedure

All participants had their blood drawn by the PI or RA on the day when they consented to participate in the study after administration of the study questionnaire. Two ml of venous blood from the cubital vein of the non - dominant hand was drawn from each of the study participants using a 18-gauge needle. The collected blood was transported to the Lancet laboratory by a trained sample motorcycle sample transporter using a cooler box for processing. This was done within a window period of one hour for all the samples.

Assessment of AMH levels for both the cancer survivors and the patients from the comparative group was carried out at the Lancet laboratory. The average turnaround time for the results was 48 hours. A copy of the results was sent to the PI via email, while a hard copy delivered to the facilities for inclusion in the participants' files for discussion in their subsequent visits.

The Lancet laboratory is a SANAs certified laboratory based in Nairobi. The laboratory uses Cobas machine to run AMH tests. The machine uses a two site ELISA (Diagnostic Systems Laboratory, Beckman-Coulter, Webster, TX). The minimum reportable concentration of this test is 0.03 ng/mL. Testing was monitored using quality control sera (two levels); with an intra-assay coefficient of variation (CV) of <6% and the inter-assay CV of <12%. The AMH results were interpreted as shown in table 8:

Table 6: *Results interpretation for the Anti Mullerian Hormone levels*

	Interpretation	Value
AMH Levels relative to AFC Response to Ovarian Stimulation	Negligible Response	<0.15
	Reduced Response	0.15 – 1.14
	Normal Response	1.15 – 2.56
	Excessive Response	>2.56

6.8.3 Quality Assurance and Infection Control

The questionnaires were pre- tested and analyzed before a final draft was administered to the study participants. The research assistants were trained on appropriate interview techniques and filling the questionnaires. Recording of clinical findings was entered after thorough scrutiny. Unique identifiers were assigned to all the study participants. If double entry was discovered, one of the questionnaires was withdrawn, discarded and serialization rectified. Information filled on the questionnaires was checked for any errors and corrected.

Study samples were taken under aseptic conditions by trained research assistants who were either qualified clinical officers or nurses. The motorbike sample transporter was trained in biosafety and appropriate handling of medical samples. Two cooler boxes were used for transporting of the sample. At the Lancet laboratory, the samples were stored and processed under strict observation of the Lancet biosafety standard operating procedures.

6.8.4 Materials

The following materials were used during the data collection process:

Table 7: *Materials as Used in the study*

Study questionnaire	Annex 3
Laboratory request form	Annex 4
Laboratory SOPs for sample processing	Annex 5
Red top plain vacutainer bottle	
2ml syringes and 18-gauge needle	
Tourniquet	
Alcohol swabs	

7.0 ETHICAL CONSIDERATIONS

Permission was sought from the KNH - UON Ethics Research Committee (ERC) to carry out this study as part of the UON thesis dissertation. Further permission was sought from the management of KNH, TCC and NRCC. Posters explaining the study procedure were placed at strategic places in the cancer clinics and information leaflets on the potential effects of chemotherapy on fertility distributed to the patients and health care workers.

All the study participants were subjected to an-opt out consenting procedure, and were only enrolled upon voluntarily signing the consent form. The procedure for blood collection was explained to the participants by the PI or RA, both in groups, and individually during the individual consenting process. No pain management medication was provided during the blood collection process. Blood samples were taken by the PI or RA; a clinical officer with training in data collection, venipuncture and good clinical practice.

The participant's personal details were de-identified by use of an assigned unique identifier, only applicable to the study. This coded information was uploaded to the excel sheet and password protected. Back up data was kept in a password encrypted external hard drive, only known to the PI. Once the results are established, they were communicated to the clinical team managing the patients for feedback during the next clinical visit and referral to a fertility expert in case the levels are below the normal. The cost for conducting the AMH levels was born by the PI.

8.0: DATA COLLECTION AND ANALYSIS

8.1 Data Variables

Table 7 shows the data variables as captured in the questionnaire for analysis:

Table 7: *Study Variables*

Socio Demographic Characteristics	Age, Parity, Last Menstrual Period, Occupation, Marital Status, Age at menarche, Age at Cancer Diagnosis[2]
Diagnosis	Type of cancer after histological diagnosis[2]
Definitive Management	Type of drugs used, Date when chemotherapy was initiated, Date when chemotherapy was completed, Number of cycles, Date when menstrual periods resumed after chemotherapy[2]
Outcome Variables	AMH levels Time to resumption of menstruation post chemotherapy[2]

2 - *This information was collected from the breast cancer patients only*

8.2: Data Collection Procedures

A paper-based interviewer administered questionnaires was administered for both breast cancer survivors and the participants in the comparative group. For breast cancer survivors, clinical information including age at diagnosis, disease stage, surgical procedures, obstetric history, radiotherapy treatments, chemotherapy regimens, and menstrual patterns before and after chemotherapy was abstracted from the patients' medical records.

The PI and the RAs worked closely with the data management teams at the three sites and abided by the laid down standard operating procedures for data handling and security. The files were retrieved from the filing area, and using a private room, had the information extracted before returning to the filing area.

The process entailed identification of participants from the three cancer centres with subsequent identification of age matched (+/- 3 years) participants from the comparable group in clinic 66. Data from the participants was collected either before the clinical review or after clinical review to minimize on the time taken at the facility. The collected data was de identified by assigning study specific unique identifiers to the study participants. A specially designed laboratory form was used for collection of data for processing of AMH levels.

8.3 Data Analysis

The study was designed to include 40 women per group to detect a mean difference of 1.2ng/ml of AMH between the survivors and the comparative group, with an 80% power and a two-sided p value of 0.05. These calculations were based on a pooled estimate of the standard deviation of 1.9 of the AMH levels from the two subsets reported as reported by Partridge et al.

The collected data was analyzed using the Statistical Package for Social Sciences Software (SPSS) Version 23. Data cleaning was done. Using the Q-Q test and a histogram, the normality test was conducted and the Levene's test for equality of variances between the two groups done. Univariate and bivariate data analysis was done for the socio demographic characteristics comparing the two groups and presented using means and standard deviations around the mean for ages, parity, BMI

and AMH levels. The Pearson's correlation model was developed to analyze the association between age, time after chemotherapy and period to resumption of menses and AMH levels. Lastly, a calculation of the proportion of patients who underwent counselling either before or after chemotherapy was done and presented as percentage. The analyzed data was visualized using pie charts, tables and graphs as presented in the results section.

9.0 RESULTS

9.1 Introduction

All the 40 patients (50%) in the comparable group were identified from the KNH clinic 66. Out of the 40 patients in the cancer treatment group 22 (28%) were from the KNH CTC, 12 (15%) from the TCC and 6 (7%) from the NRCC. The study was conducted between June and September 2019.

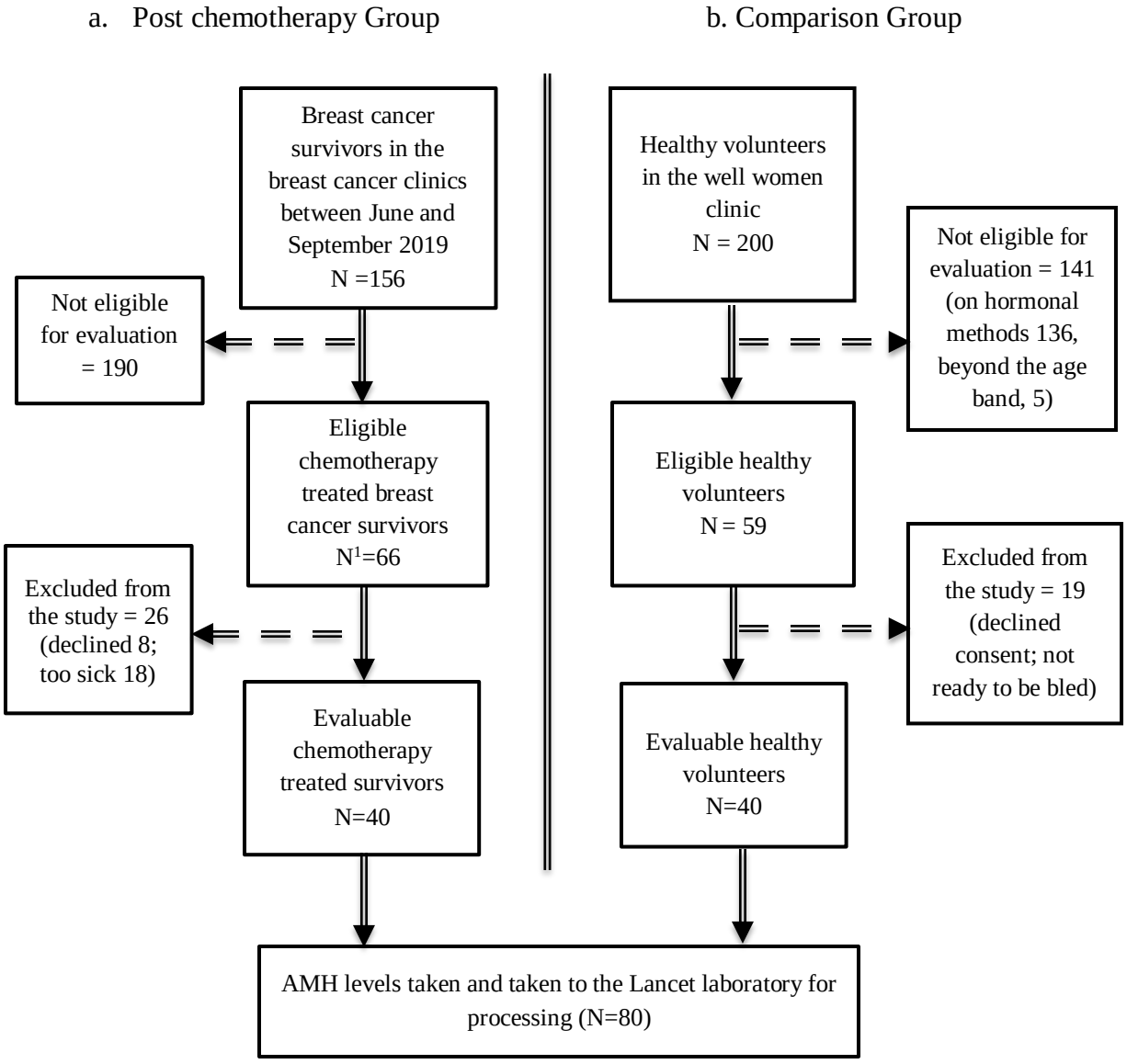

Figure 8: *Study Flow *No Sample was Rejected by the Lancet Laboratory: Results for all the 80 Samples were Received for Analysis*

9.2 Socio Demographic and Clinical Characteristics of the Study Participants

Table 1 shows the socio demographic characteristics of the study participants. The mean age and BMI for the chemotherapy group were slightly higher that for women attending clinic 66 (39 vs 34, f=0.176, p=0.672; 30.74 and 28.34, f=0.002, p 0.964; 14.28 vs 13.90, p, 0.230 respectively). The mean age at diagnosis of breast cancer among the cancer survivors was 36 years (s.d 5.07).

The level of education was disaggregated into primary, secondary and tertiary, with a majority of the women across the two groups having attained tertiary level of education (18, 45% and 24, 60% respectively). Across the two groups, married women formed the bulk of the study participants at 80% and 85% for the treatment and comparison group respectively (p,0.79), just as the number of women who had at least one child at the time of data collection (37, 92.5 and 38, 94.9% respectively, p, 0.5). This was not however statistically significant (p,0.644).

Table 9: *A Comparison of the Socio Demographic and Gynecological Characteristics of the Study Participants*

Characteristic	Cancer Treatment Group (n=40)	Comparison Group (n=40)	P Value
Means			
***Age (SD)**	39 (3.64)	34 (5.96)	0.672
***BMI (SD)**	30.74 (6.41)	28.34 (7.45)	0.964
Level of Education			
Primary	09 (22.5)	06 (15.0)	0.397
Secondary	13 (32.5)	10 (25.0)	
Tertiary	18 (45.0)	24 (60.0)	
Marital status			
Single	06 (15.0)	05 (12.5)	0.79
Married	32 (80.0)	34 (85.0)	
Separated	02 (05.0)	01 (02.5)	
Parity			
Zero	03 (07.5)	02 (05.0)	0.644
At least one	37 (92.5)	38 (95.0)	

***Levene's test** for equality of variances, f for age =0.176 (p,0.672) and for BMI = 0.002 (p, 0.964)

9.3 Clinical Characteristics of the Participants in the Cancer Treatment Group

Out of the 40 participants, 32 (80%), reported having had regular monthly periods before treatment, with the rest, 8 (20%) experiencing irregular menses. During the period of chemotherapy, 31 (77.5%) developed amenorrhea; this number however reduced to 13, with 18 (58%) resuming their menstruation within an average period of 8.2 months (s.d. 6.95). The average

time since receiving the last chemotherapy was 2.6 years (s.d 2.17, min 1 year, max 8 years). One of the participants (97.5%) had HIV while 3 had hypertension and on follow up

The histological diagnosis had clearly been documented in 35 records as shown in figure 10. The most common histological diagnosis for breast cancer was invasive ductal carcinoma (29, 83%), followed by invasive lobular carcinoma at 5% (2 participants).

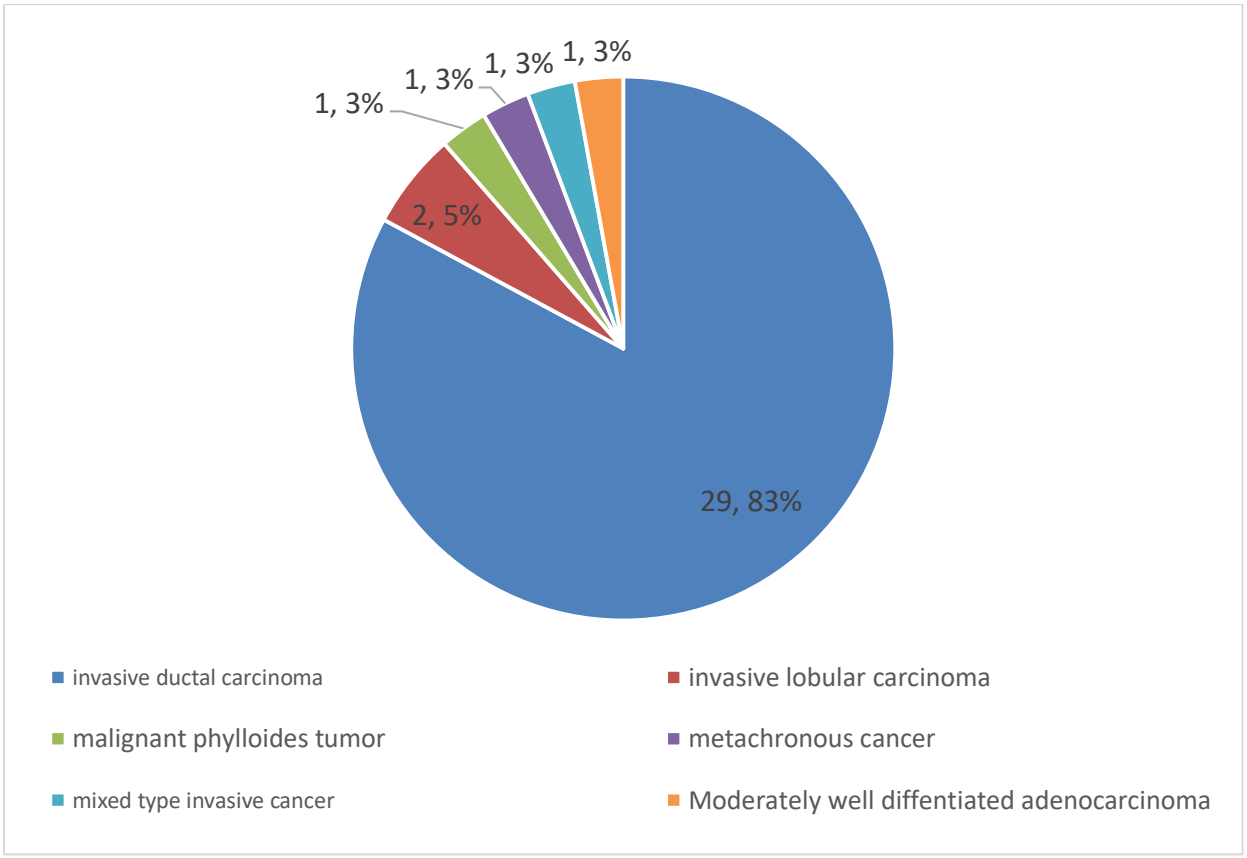

Figure 9: *Histological Diagnosis of the Breast Cancer Survivors attending the Breast Cancer Clinics at the three study sites, n=35*

The pie chart below shows the different regimen types as used for the treatment of breast cancer with a majority of the patients (16, 308%) being on the Adriamycin, Cyclophosphamide and Paclitaxel combination.

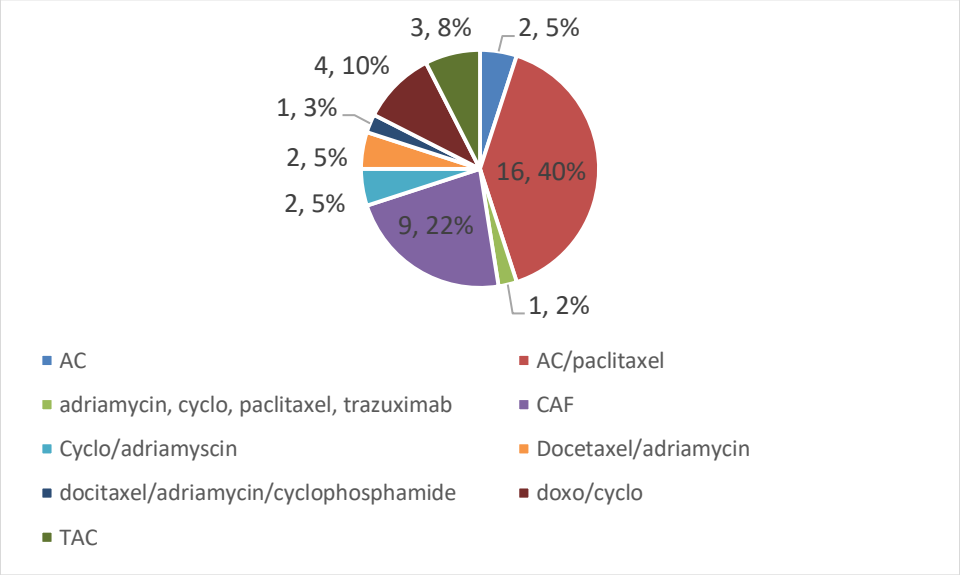

Figure 10: *Comparison of the Anti-Mullerian Hormone levels among Participants in the Breast Cancer Treatment Group and those in the Comparison Group*

The Mean AMH levels across among the breast cancer treatment group and the comparison group was 0.190 (s.d 0.253) 2.105ng/ml (s.d 1.967) respectively. There was a statistically significant difference between the mean AMH levels among the participants who had been treated for breast cancer for at least one year compared to those who had not been exposed to chemotherapy (p, <0.0001).

Further classification of the levels of AMH across the two study groups was done as presented in the table 3. All the 40 participants in the cancer treatment group exposed to chemotherapy, had

AMH levels that would have negligible (27, 67.5%) or reduced (13, 32.5%) response to ovarian stimulation. Among the comparable group a total of 12 participants expressed AMH levels that were reduced (09, 22.5%) or negligible (03, 7.5%) to ovarian stimulation, translating to 30% of patients with sub optimal ovarian function.

Table 10: *Classification of Anti Mullerian Levels, Comparing the Participants who had been exposed to Chemotherapy with those in the Comparison Group*

	Response (ng/ml)	**Chemotherapy Exposed Group (n=40)**	**Comparison Group (n=40)**
AMH Levels relative to AFC Response to Ovarian Stimulation	Negligible Response (<0.25)	27 (67.5)	03 (07.5)
	Reduced Response (0.15-1.14)	13 (32.5)	09 (22.5)
	Normal Response (1.15 – 2.56)	0	17 (42.5)
	Excessive Response (>2.56)	0	11 (27.5)

P, <0.001

Since none of the patients in the chemotherapy exposed group had AMH levels above those associated with a normal ovarian stimulation response, the *Haldane-Anscombe* correction was done before comparing the two groups. The risk of developing poor ovarian response to stimulation as assessed by the AMH levels was 3.25 time more among patients who had undergone treatment for cancer compared to those who had not been exposed to the chemotherapy agents, (95% CI, 2.02 to 5.23, p<0.0001).

9.5 Correlation of the Clinical and Socio Demographic Characteristics of the Study Participants

A correlation of age with the AMH levels was done across the two groups; among participants who had not been exposed to chemotherapy, there was a moderately strong negative correlation

between age and the AMH levels, with participants with a higher age having lower levels of AMH as shown in figure 11 (Pearson test of -0.369, p value 0.047).

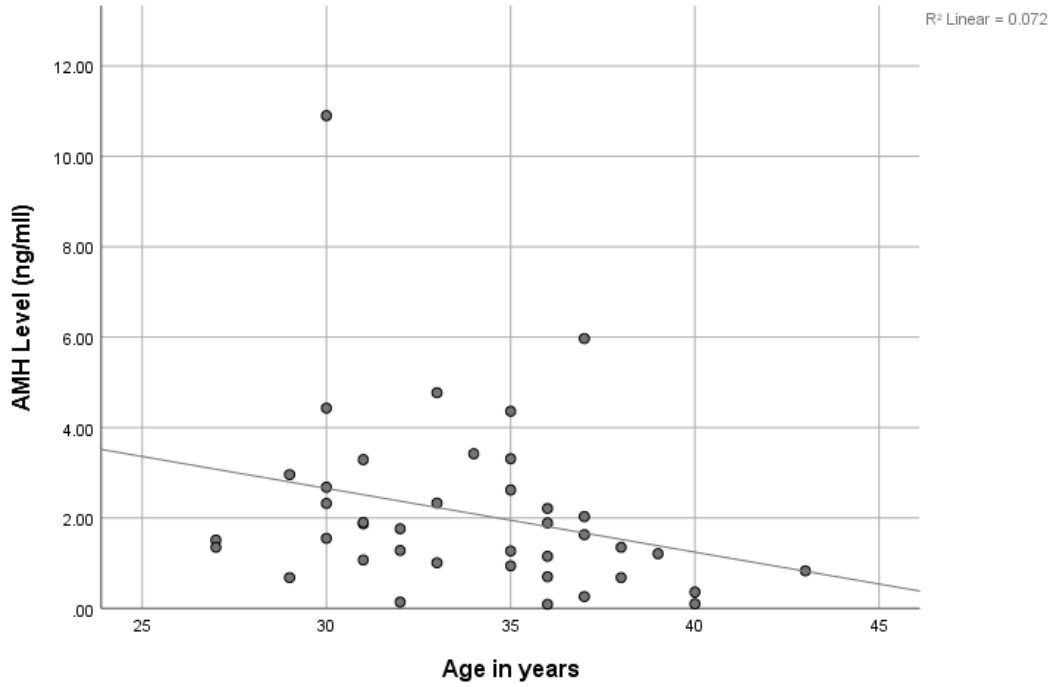

Figure 11: *Correlation of the Participants in the Non-Chemotherapy Exposed Group with the Anti Mullerian Hormone Levels*

A similar observation though with a stronger negative correlation was observed among patients who had been exposed to chemotherapy as shown in figure 12 (Pearson test of -0.440, p value <0.0001).

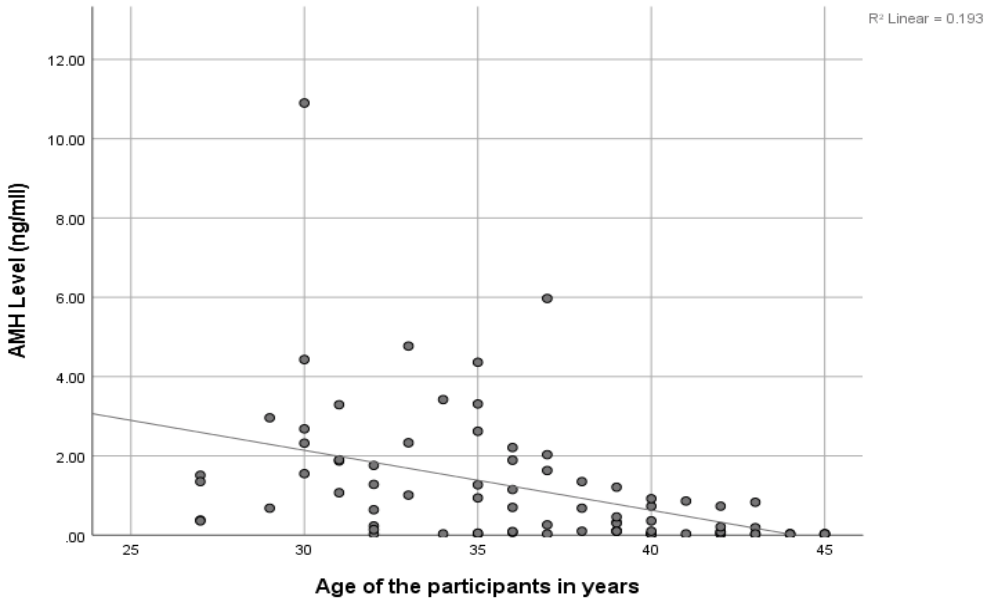

Figure 12: *Correlation of Age among Women who had Been Exposed to Chemotherapy and Anti Mullerian Hormone Levels*

Among the patients who had been exposed to chemotherapy, a correlation of time since the last chemotherapy with the Anti Mullerian Hormone levels was done. There was a moderately strong positive correlation between AMH levels and the time in years since receiving the last chemotherapy (Pearson test of 0.427, p value = 0.007), with patients who had recently been on treatment recording lower levels of AMH.

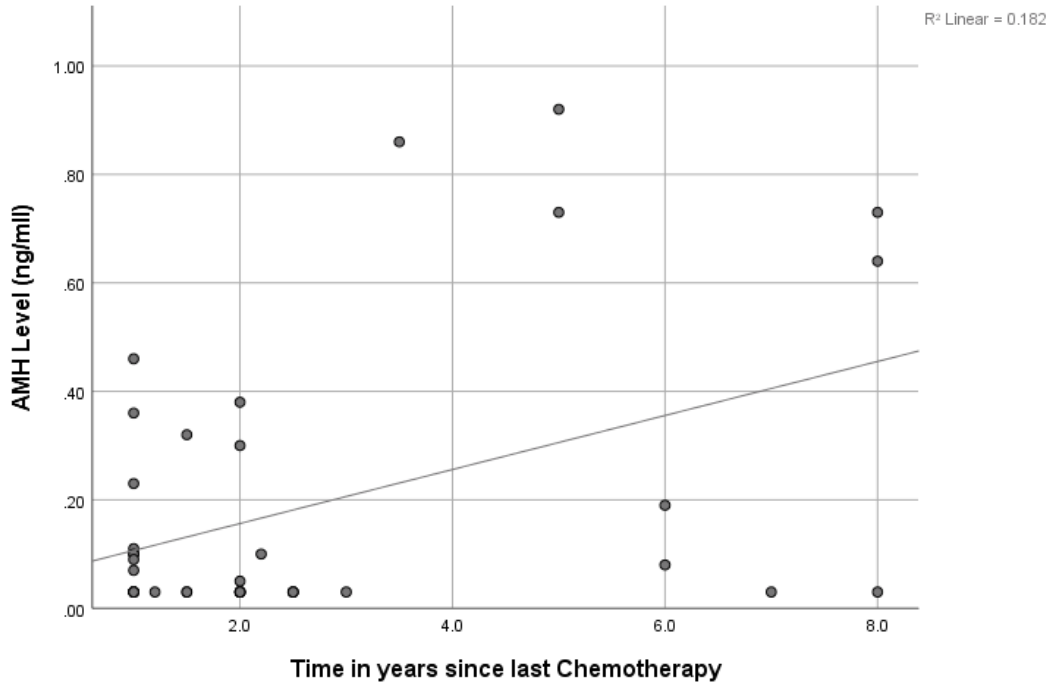

Figure 13: *Correlation of Anti Mullerian Hormone and Time in Years since Last Chemotherapy*

Further correlation study was done among the 22 patients who reported resumption of their menses. There was a moderately strong negative correlation between AMH levels and time taken to resume menses (Pearson correlation, -3.20). This was not however significant statistically (p, 0.119).

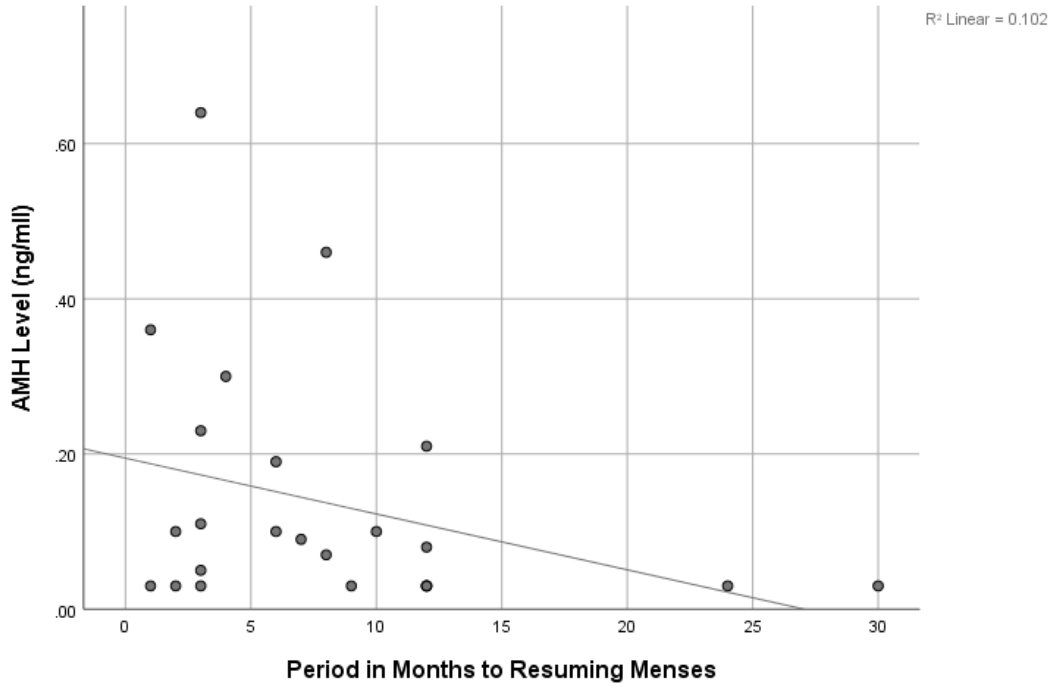

Figure 14: *Correlation of Anti Mullerian Hormone Levels and Period in Months to Resumption of Menses*

There was a moderately strong positive correlation between the age of the patient and the time taken to resume the monthly periods with older patients taking longer to resume their periods (Pearson's Correlation of 0.532). This finding was significant statistically, p=0.006

5.6 Secondary Objective: Counselling on Fertility and Utilization of a Fertility Assessment Tool

Only 40% of the participants had a discussion about their fertility with their physician; none of the health facilities where the study was undertaken had a tool or protocol for assessing fertility before or after treatment for breast cancer.

6.0 DISCUSSION

The study was among the very few studies on the use of AMH to assess ovarian reserve among pre-menopausal breast cancer survivors in Africa, and the first one to be done in Kenya. The results, confirmed the findings from other studies done in other developed countries where AMH has been used to accurately estimate ovarian reserve in premenopausal recipients of chemotherapy for breast cancer (14).

The mean age for the post chemotherapy exposed participants in our study was 39 years. This is comparable to the study done by Anderson et al, where the mean age for the post chemotherapy exposed group was 41 years; in both studies, the comparable group included age matched women without exposure to chemotherapy and with confirmed fertility. This was in keeping with our objective, which was to evaluate OR in young women with breast cancer using AMH levels as a biomarker.

A majority of the patients in the study (80%) had their cyclic menses. Although a considerable number, 31 (77%) had undergone a variable period of amenorrhoea following chemotherapy, clinical characteristics alone (patient age, BMI and parity) were not found to be discriminatory once cyclical activity resumed. This conforms with the generally accepted notion that clinical characteristics are not reliable estimates of reproductive age. The two groups were comparable in the socio demographic characteristics (Levene's test=, $p>>0.05$).

The patients had undergone a work up for histological diagnosis with the commonly diagnosed cases being invasive ductal carcinoma (29, 83%). The ESMO guidelines recommend the use of cyclophosphamide-based regimen as first line therapy for patients with breast cancer (cite). This

however has been associated with poor ovarian response (16) compared to patients who use newer regimens such as taxanes (16). In our study, all the 40 post chemotherapy participants had at some point been exposed to an average of 6 doses of cyclophosphamide-based regimen, using the ESMO guidelines.

The Mean AMH levels across among the breast cancer treatment group and the comparison group was 0.190 (s.d 0.253) 2.105ng/ml (s.d 1.967) respectively. There was a statistically significant difference between the mean AMH levels among the participants who had been treated for breast cancer for at least one year compared to those who had not been exposed to chemo-radiotherapy (p, <0.0001).

Our findings are similar to those by Lutchman et al in London, where 22 age matched breast cancer survivors aged 22 to 44 years had their basal AMH and basal inhibin B levels assessed pre and post chemotherapy (17). The AMH levels in this study were much higher in patients tested pre-chemotherapy compared with post-chemotherapy, although the findings were not statistically significantly (4.471.1 vs 0.0770.02, P,0.05).

Age has been shown to be a major influencer of AMH levels, with most studies indicating older age being associated with lower levels of AMH (18). Our study confirmed this observation across the two groups (moderately strong correlation of age and AMH levels, Pearson's test -3.69, p, 0.047 among the non-chemotherapy exposed group and a stronger negative correlation of -0.440, p <0.001, among post chemotherapy participants).

The time taken for the recovery of the ovary following exposure to chemotherapy is variable. Ovarian injury is a function of the type of chemotherapy agent, the duration and dosage of exposure and the age of the patient. In our study, there was a moderately strong positive correlation of time since the last chemotherapy was administered and the levels of AMH levels (Pearson test of 0.427, p value = 0.007).

As a secondary finding, study aimed at assessing whether patients on follow up for breast cancer usually undergo counselling about the effect of the chemotherapy on ovarian function and subsequent fertility. Only 40% of the participants had a discussion about their fertility with their physician. Furthermore, none of the health facilities where the study was undertaken had a tool or protocol for assessing fertility before or after treatment for breast cancer.

In summary, this study confirms the use of AMH as a biochemical parameter of ovarian reserve assessment in patients with breast cancer. The cohort was disease standardized where patients received cyclophosphamide-based chemotherapy. These results taken together support the hypothesis that ovarian reserve is reduced in women in the pre-menopausal period following treatment with chemotherapeutic agents for breast cancer. The data also add to the understanding of the pathophysiological processes involved.

6.1 Conclusion and Recommendations

These findings have major implications for breast cancer survivors, for whom reproductive issues are a major concern (1). Furthermore, the potential exists for ovarian reserve testing to be applied in patients with other types of cancer. To achieve this, a large sample size should be followed up

longitudinally to determine the potential therapeutic role of ovarian reserve testing in this cohort. In conclusion, this study confirms that ovarian reserve can be assessed in breast cancer patients using AMH to inform patients on their prospects of fertility treatment post chemotherapy.

6.2 Study Results Dissemination Plan

The results of the study were presented to the department of Obstetrics and Gynecology for inputs from the faculty and as part of the fulfillment of the master in Obstetrics and Gynecology. Following the revisions by both the internal and external examiners, the findings were disseminated to the KNH and Texas Center Cancer clinics and the KNH clinic 66, in form of CMES and a report submitted to the hospital management. Furthermore, the results from the participants were incorporated into the clinical management system for individual discussion with the patients during their subsequent clinical follow up visits.

6.3: Study Limitations and how to minimize them

In view of the various factors influencing the levels of AMH in females of reproductive age being an independent factor, there is likely to be confounding. To overcome this, matching for age at the point of selection of the study participants was done. In addition, patients with conditions that are associated with ovarian dysfunction were excluded from the study.

The study entailed collecting historical information about the pre-chemotherapy interventions such as age at menarche. As a result, the participants may not recall well these events. This information was correlated with the information in the patients' files as a way of confirmation.

In addition, some information is likely to be missed from the patients' files. Where essential data was missing, the PI picked the next eligible file for enrolment in to the study; this decision was made on a daily basis.

Lastly, due to the prohibitive cost of performing the AMH test, pretreatment measurements of serum AMH was be performed. This could have been useful for comparison as demonstrated by Dillon et al (38) where the pretreatment serum AMH was significantly associated with post-treatment serum AMH. To overcome this, a nomogram from the age matched comparative group was used to compare the AMH levels.

6.4: Study closure and plan

The study was conducted in three phases: phase one entailed recruitment and data collection, followed by data analysis and presentation to the department of obstetrics and gynecology for review. The third phase entailed feedback to the key stakeholders. The recommendations from these feedback sessions were incorporated in to the final report before publication.

6.5 Study Timelines

	Jan 2018	Mar 2018	Sep 2018	Dec 2018	Jan 2018	Feb 2018	Mar 2019	Apr 2019
Concept development								
Proposal development								
Ethical approval								
Data collection								
Data analysis								
Results presentation, dissemination and close out								

7.0: ANNEXES

Annex 11.1: Consent Form

Informed consent form for the study on 'Comparative evaluation of ovarian reserve one year following cytotoxic therapy for breast cancer in selected sites in Nairobi Kenya'

Name of Principal Investigator: Dr Chrisostim Barasa

Name of Organization: University of Nairobi, Department of Obstetrics and Gynecology

Name of Sponsor: Kenyatta National Hospital, Texas Hospital

This informed consent form has two parts:

- Information sheet (to share information about the study with you)
- Certificate of consent (for a signature if you choose to participate)

Part I: Information Sheet

Introduction

I am……..……a medical doctor undertaking studies at the University of Nairobi, department of Obstetrics and Gynecology. I am conducting a study on 'Comparative evaluation of ovarian reserve one year following cytotoxic therapy for breast cancer at the KNH and Texas cancer hospitals'. I am going to give you information about this study and invite you to participate in the study. Before you decide, you are free to ask for clarifications. This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, please feel free to ask.

Purpose of the research

Many studies about the effect that treatment of patients with breast cancer has on their ability to deliver have been done in the developed countries. Not much information however exists about this, especially in developing countries, despite the fact that an increasingly larger proportion of women are getting diagnosed with breast cancer at an earlier age. In addition, due to better medicines, more and more breast cancer survivors are living longer and will at some point desire to sire children.

In order for us to establish this effect among the population in developing countries, we have designed a study where data will be collected from female breast cancer survivors attending clinic at either the Kenyatta National Hospital or the Texas Hospital. For us to be able to compare the levels of a substance that is used to measure the level of ovarian function among women, we will also get information from women who have not been affected by cancer of the breast or any other cancers. This data will be collected from women attending the well woman clinic 66 at the Kenyatta National Hospital.

The ultimate aim of our study is to find out the extent to which treatment for breast cancer affects the ovaries. This information will be helpful in further designing treatment protocols that are friendlier to the women who desire to sire children in future and counsel those who may not be able to sire children after the treatment.

Type of Intervention

This study will involve your participation as an individual. It will take about 20minutes. In addition, 2mls of blood sample will be taken from your non dominant hand for assessment of a hormone that is

used to measure how well your ovaries are functioning; this hormone is called Anti Mullerian Hormone.

Participant Selection

You are being invited to take part in this study either as a breast cancer survivor following intent to treat chemotherapy for breast cancer at least one year ago or as a participant in the comparative group to provide us with information about yourself and on the effect that the treatment of the cancer may have had on your ability to sire children.

Voluntary Participation

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate all the services you receive during the follow up clinics at the cancer centre or clinic 66 will continue and nothing will change.

Procedures

We are inviting you to take part in this research project. If you accept, you will be asked to respond to a few questions that the research assistant/I will administer to you. You will be asked a few questions by the research assistant or myself. If you do not wish to answer any of the questions included in the study, you may skip them and move on to the next question. The information recorded is confidential, your name is not being included on the forms, only a number will identify you, and no one else except our data analyst will have access to the data.

Duration

The data collection process for the study will take place over a period of three months. During that time, we will visit you once for interviewing and collection of the blood sample. Each interview will last for about 20.

Risks

In case in the course of the survey you feel uncomfortable talking about some issues, you are at liberty not to answer any question or take part in the discussion; that is also fine. You do not have to give us any reason for not responding to any question, or for refusing to take part in the study. In addition, a blood sample will be taken by performing a needle prick on the front part of your non dominant arm. This may cause some pain, but it is manageable, without necessarily needing any pain killers. If this is not tolerable too, kindly feel free to let us know and pull out of the study.

Benefits

The results of the assessment of the ovarian function will be shared with the clinician taking care of you and discussed with you. Based on the outcomes, you will be referred to a fertility expert of your choice for further evaluation. In addition, your participation is likely to help us find out more about how to improve the provision of fertility preservation services before administration of chemotherapy to patients with cancer. Referral, free of charge

Reimbursements

You will not be provided with any incentive to take part in the study.

Confidentiality

The study will be conducted from a private room and from the clinic where you routinely attend follow up clinics for the cancer management or receive your routine well woman services. We will not be sharing information about you to anyone outside of the study team. The information that we collect from this research project will be kept private. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key.

Sharing the Results

Nothing that you tell us today will be shared with anybody outside the research team, and nothing will be attributed to you by name. The knowledge that we get from this research will be shared with you and the hospital where you attend the clinic before it is made widely available to the public. Each participant will receive a summary of the results. There will also be small meetings in the clinics to share the outcomes of the study; these will be announced. Following the meetings, the results will be presented at the department of Obstetrics and Gynecology, University of Nairobi as part of the fulfilment of the master of Obstetrics and Gynecology. Subsequently, the results will be published so that other interested people may learn from the study.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so, and choosing to participate will not affect your access to the services offered at the cancer clinic.

Who to Contact

If you have any questions, you can ask them now or later. If you wish to ask questions later, you may contact the principal investigator on the following number: Dr Barasa on 0722419651/0782419651 and KNH-ERC on 0799495829. This proposal has been reviewed and approved by the Kenyatta National Hospital – University of Nairobi Ethics Review Committee; the committees' task it is to make sure that research participants are protected from harm.

Part II: Certificate of Consent

I have been invited to participate in the study on '**Comparative evaluation of ovarian reserve one year following cytotoxic therapy for breast cancer at the KNH and Texas Cancer hospitals'**. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

Print Name of Participant_____

Signature of Participant _____

Date _____ (Day/Month/Year)

If not able to read and write,

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of person taking the consent_____

Signature of person taking the consent_____

Date _____ (Day/Month/Year)

Swahili version of the consent form

Fomu za idhini kwa kujihusisha kwa utafiti kuhusu saratani ya akina mama na madhara ya madawa za kutibu saratani kwa kizazi katika hospitali kuu jijini Nairobi Kenya

Jina la mpelelezi mkuu: Dkt. Chrisostim Barasa

Jina la shirika: Chuo kikuu cha Nairobi

Jina la mdhamini: Hospitali kuu ya Kenyatta

Hii fomu ya idhini ina sehemu mbili:

- Karatasi ya habari (kueleza habari kuhusu utafiti nawe)
- Cheti cha idhini (saini iwapo unachagua kushiriki)

Sehemu ya kwanza. **Karatasi ya habari**.

Dibaji

Mimi ni……..nafanya kazi na mwenzangu Dkt Chrisostim Barasa, aliye mwanafunzi katika chuo kikuu cha Nairobi. Tunafanya utafiti kuhusu ugonjwa wa saratani kwa akina mama, na madhara ya kutumia madawa ya kutibu ugonjwa huu, hasaa kwa kizazi cha akina mama. Nitawapa maelezo na kuwaaalika kuhudhuria katika utafiti huu. Kabla ya kuamua, uko na uhuru wa kuulizia ufafanuzi zaidi kutoka kwa yeyote kwa starehe zako. Fomu hii ya idhini/ ridhaa huenda ikawa na maneno ambaye huyaelewi. Tafadhali niulize usipoelewa tunaavyopitia habari name ntachukua muda wangu kukueleza. Vilevile, kama una maswali baadae waezaniuliza mimi, ama wenzangu hapa.

Madhumuni/ nia ya utafiti

Ugonjwa wa saratani sanasana hutibiwa kutumia dawa ambazo zaweza sababisha madhara kwa miili ya wanadamu. Sehemu ambazo huadhiriwa kwa urahisi ni kama sehemu za uzazi za akina mama (ovary); hizi sehemu za uzazi zinapodhuruwa, inaweza sababisha mama kuwa tasa ama

kutofuja hevi. Kwa sababu hii, na ili tuweze kujua kiwango cha madhara haya kwa miili zenu, tungependaa kupata mbinu za kuboresha huduma ambazo tunapeana. Tunaamini unaweza kutusaidia kwa kutuambia kile ujuacho kuhusu hali kawaida za afya na vianzo vinavyochangia upatikanaji wa huduma hizi.

Aina ya kuingilia kati

Huu utafiti utahusisha ushiriki wako kibinafsi. Utachukua dakika ishirini tu.

Uchaguzi wa mshirika

Unaalikwa kushiriki katika huu utafiti kwasababu tunahisi kama wakuu wa wanachama nyumbani kuwa wawezachangia mengi kwa kuelewa kwetu na kujua mazoea ya afya mtaaani.

Ushiriki wa hiari

Ushirika wako katita utafiti huu ni kwa hiari yako mwenyewe. Ni chaguo lako kushiriki au kutoshiriki. Ukichagua kutoshiriki, huduma zote unazopokea kliniki ya mkononi zitaendelea, hakuna kitakachobadilika.

Utaratibu

A. Tutakuuliza utusaidie kujua zaidi kuhusu juu ya afya yako baada ya kupata tiba ya saratani. Tunakualika kujiunga na hi mpango huu wa utafiti. Ukikubali, utaulizwa kujibu maswali machache nitakayokuuliza.

B. Utajaza fomu ya utafiti ambayo nitapeana ama mwenzangu na tutasanya au waweza kujibu dodoso wewe mwenyewe, ama ukisomewa na unawezasema kwa sauti jibu unalotaka niandike

chini. Kama hautaki kujibu swali lolotekatika utafiti,wawezaenda kwa swali jingine. Habari itakayopeanwa ni siri, jina lako halitanakiliwa kwa fomu, ni baaadhi tu ambao watakutambua na hakuna mwengine ila mkaguzi wa takwimu (data) atakayeafikia utafiti wako.

Hatari

Japo kwa katika utafiti hutahisi vizuri kuongelea swala Fulani, hautalazimishwa kujibu swali lolote au kujihusisha na majadiliano/mahojiano/utafiti kama huhisi kufanya vile na ni bora pia. Huna lawama ya kutupatia sababu ya kutojibu swali lolote lile, au kwa kukataa kujihusisha na utafiti.

Faida

Hakutakua na faida za moja kwa moja kwako wewe lakini kuhudhuria kwako huenda kukatusaidia kujua mengi kuhusiana na jinsi ya kuboresha kupeana huduma za afya katika jamii yako.

Kulipia

Hutapewa malipo ya na mna yoyote kuchangia utafiti.

Siri

Huu utafiti utakavyofanyika katika jhamii huenda ikavutia watu na iwapo uutahudhuria,wawezaulizwa maswali na baadhi ya watu katika jamii. Hatutapeana habari habari kukuhusu nje ya kundi letu. Habari ambayo tutachukua kutokana na huu utafiti itaekwa kibinafsi. Habari yoyote kukuhusu itakuwa na nambari badala ya jina lako. Watafiti pekee ndio watakaojua nambari yako na haitafikiwa na yeyote tu.

Kugawana matokeo

Hakuna kile utakachotwambia kitajadiliwa na yeyote yule nje ya kundi hili la utafiti, na hakuna kitakachoidhinishwa jina lako. Ujumbe ambao tutapata kutokana na huu utafiti tutajadili nawe na jamii kabla iwe huru kwa watu wengine. Kila atakayeshiriki atapokea maelezo kiufupi ya majibu. Pia kutakua na mikutano ndogo katika jamii na hii itawasiliswha kutokana na mikutano, tutachapisha majibu ndiposa wengine walio na na hamu waweze kujifunza kutokana na utafiti.

Haki ya kukataa au kujitoa

Sio lazima ushiriki katika utafiti huu kama huna nia ya kufanya hivo, na kuchagua kutoshiriki haitadhuru kuopokea huduma zinazopeanwa katika kliniki za mkononi kwa njia yoyote ile.

Wa kuwasiliana nao

Ikiwa una swali lolote, unaweza ukauliza sasa hivi ama baadae. Kama una nia ya kuuliza baadae unawezawasiliana nasi kupitia wafwatao: Dkt. Barasa kwa 0722419651 ama KNH-UON ERC kwa nambari 0799495829 Hili pendekezo la utafiti limepitiwa na kukubaliwa na bodi ya chuo kikuu cha Nairobi pamoja na Hospital kuu ya Kenyatta; chanzo chao ni kuhakikisha kwamba washiriki wa utafiti wanalindwa kutokana na madhara.

Sehemu ya pili: Cheti cha idhini/ridhaa

Nimealikwa kushiriki katika utafiti kuhusu mambo yanayochangia upatikanaji wa huduma za afya. Nimepitia habari ifuatayo, ama nimesomewa. Nimekua na nafasi kuuliza maswali kuhusu kuihusu na maswali yoyote ambayo nmeulizwa nimeyajibu kadri ya ufahamu na utoshelezi wangu. Ninaidhini kibinafsi kuwa mshirika katika stadi hii.

Jina la uchapishaji la mshirika……

Saini ya mshirika……..

Tarehe ……

Iwapo huna elimu

Nimehudhuria usomaji kamili wa fomu ya idhini kwa huyu mshirika, naye amekua na nafasi ya kuuliza maswali. Nina uhakika kuwa huyu amepeana idhini pasipo kulazimishwa.

Jina la uchapisho la shahidi…… alama ya kidole ya mshirika……..

Saini ya shahidi…….

Tarehe ……

Wasilisho la mtafiti/mwenye kuchukua idhini.

Nimemsomea kitaratibu karatasi ya habari huyu mwenye uwezo wa kushiriki, na kwa kadri ya uwezo wangu nimehakikisha kwamba huyu mshirika anaelewa kuwa yafuatayo yatafanyika:

1.

2.

3.

Nathibitisha kuwa mshirika alipewa nafasi ya kuuliza maswali kuhusu utafiti huu, na maswali yote aliyouliza mshirika yamejibiwa kisawasawa na kwa kadri ya uwezo wangu. Nathibitisha kwamba huyu hajalazimishwa kupeana idhini na idhini imepeanwa bure na kwa kujitolea.

Jina la uchapisho la anayechukua idhini……..

Saini ya anayechukua idhini……..

Tarehe …….

Annex 11.2: Study Questionnaire

Study Title: Comparative evaluation of ovarian reserve one year following cytotoxic therapy for breast cancer at the Kenyatta National Hospital and Texas Cancer Center

Date:……..

Time:…….

Section I (to be administered to all the study participants by the research assistant; kindly tick the boxes as appropriate)

1. Serial number……..

2. Study group *(tick as appropriate)*

   ☐ Cancer Survivor group

   ☐ Comparison group

3. What is your age in years……

4. Indicate weight in Kg……

5. Indicate height in cm…….

6. Calculated BMI…….

7. What is your highest attained level of education *(tick as appropriate)*

   ☐

Primary

☐ Secondary

☐ Tertiary (College/University)

8. How many children do you have? Have you lost any pregnancies? If so, how many?

……..

(Indicate as para…..+……)

9. Marital status

☐ Single (never married)

☐ Married

☐ Divorced/Separated

10. What was your age when you started experiencing your monthly periods? ……

11. Do you know your HIV status? Kindly share with us in case comfortable

☐ Positive

☐ Negative

Section II (this section is to be administered to the breast cancer survivors only)

12. How was your menstrual frequency before chemotherapy

☐ Regular

☐ Irregular

☐ Amenorrhea

13. How was your menstrual frequency during chemotherapy

☐ Regular

☐ Irregular

☐ Amenorrhea

14. How is your menstrual frequency after chemotherapy

☐ Regular

☐ Irregular

☐ Amenorrhea

15. Did you experience any interruption of menses after chemotherapy? *(for the cancer survivors group)*

☐ Yes

☐ No

If yes to 10 above for how long did it take before you resumed your menses? *(indicate in days)*

……

16. Was the following done during the preparation for chemotherapy?*(indicate in months)*

a. Physician discussed fertility issues

☐ Yes

☐ No

b. Physician used checklist to assess fertility

☐ Yes

☐ No

c. Referred to fertility expert for fertility assessment

☐ Yes

☐ No

17. Was the following done during the preparation after chemotherapy? *(indicate in months)*

a. Physician discussed fertility issues

☐ Yes

☐ No

b. Physician used checklist to assess fertility

☐ Yes

☐ No

c. Referred to fertility expert for fertility assessment

☐ Yes

☐ No

18. Did the treatment for cancer make you stop receiving your monthly periods?

☐ Yes

☐ No

If yes to question 13 above, how long was the interruption?

……

19. Did any of the health care workers discuss with you or refer you to a fertility specialist after treatment with chemotherapy?

☐ Yes

☐ No

20. The PI/Research Assistant to obtain the following information from the participant's file/records:

Date when cancer was diagnosed (histological diagnosis)	
Time since the last chemotherapy was administered	
Histological diagnosis	
Cancer stage at time of diagnosis	
Comorbidities	
Surgery Type of surgery Date when done	
Chemotherapy Regimen type Age when initiated Date when initiated	
Number of cycles	
Date when finished chemotherapy	
Number of cycles attended	

Annex 11.3: Budget

Category/item	**Cost in Ksh**
Charges for KNH/UoN ERC proposal review	3,000
Sample processing @Ksh 4,899	558, 486
Research assistants @Ksh 25,000	50,000
Data entry	5,000
Statistician	50,000
Photocopying/printing and publishing	20,000
Miscellaneous	10,000
Total	**696,486**

Annex 11.4: Laboratory request form

Patient's name:

Patient's unique number:

Patient's phone number:

Patient's age:

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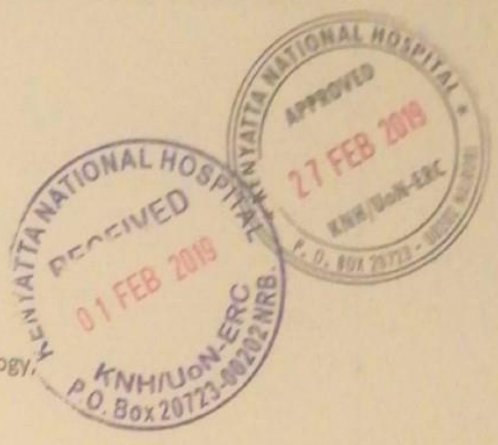

Thru'

Prof Omondi Ogutu,

Chairman, Department of Obstetrics and Gynecology,

University of Nairobi.

Dear Professor M.L Chindia

REF: CORRECTIONS FOR THE STUDY ON ' COMPARATIVE EVALUATION OF OVARIAN RESERVE ONE YEAR FOLLOWING CYTOTOXIC THERAPY FOR BREAST CANCER AT THE KENYATTA NATIONAL AND TEXAS CANCER HOSPITALS' (P820/20/2018)

This is to acknowledge receipt of your letter dated 14th January 2019, with corrections to my proposal as earlier on forwarded to the KNH-UoN committee for review. Here in attached, kindly find THREE (3) copies of revised proposal as guided in the table below:

	Observation/Suggestion	Correction done	Page
1	Abstract: Remove statement on expected outcome and add study utility section instead	Done	11
2	Specific objective 3: with the current sample size will you have the power to meet this objective?	Recalculated: with a power of 80%; new sample size recalculated to 86 breast cancer survivors; two main regimens used in the management of patients with breast cancer, therefore able to achieve the minimum of 30 patients for comparison	33
3	Study site: Check missing information on Texas Cancer Hospital	Information included	32
4	Study procedures i. Laboratory procedures – describe in full ii. Data abstraction from medical records – describe in full	 Done Done	 37 37,38
5	Consent document i. Revise to simplify language and understanding ii. Provide KNH-UoN ERC contacts	 Done Done	 43,44 46
6	Study questionnaire: include study title, date and instructions	Done	52

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Ref: KNH-ERC/A/364

30th September, 2019

Dr. Nasra Jattani Boru
Reg. No. H58/88984/2016
Dept. of Obstetrics and Gynaecology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Nasra

RESEARCH PROPOSAL: BURDEN MANAGEMENT AND OUTCOMES OF CANCER OF VULVAR AT THE KENYATTA NATIONAL HOSPITAL (P458/06/2019)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 30th September 2019 – 29th September 2020.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

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The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
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