SERUM ANTI-MULLERIAN HORMONE AS A PREDICTOR OF METAPHASE II OOCYTE YIELD DURING CONTROLLED OVARIAN STIMULATION IN A PRIVATE FERTILITY CLINIC IN NAIROBI, KENYA.

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DECLARATION

This research work and dissertation is my original work and to the best of my knowledge it contains no materials previously published or written by another person. It has not been submitted for award of a degree in any other university. References to work done by others have been clearly indicated.

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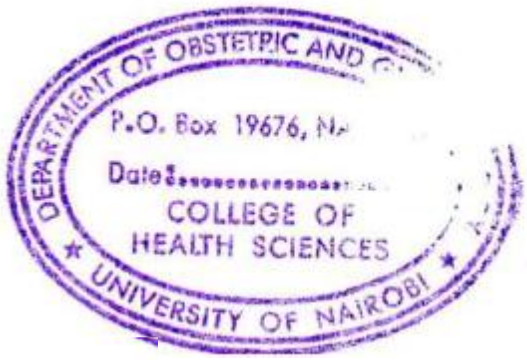

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ABBREVIATIONS

AFC	Antral Follicular Count
AMH	Anti-Mullerian Hormone
ART	Assisted Reproductive Technology
CPR	Continuing Pregnancy Rates
COC	Combined Oral Contraceptives
ERC	Ethical Review Committee
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
HCG	Human Chorionic Gonadotrophin
iCOS	Individualized Controlled Ovarian Stimulation
ICSI	Intracytoplasmic Sperm Injection
IVF	In Vitro Fertilization
KNH	Kenyatta National Hospital
LBR	Live Birth Rates
MII	Metaphase II oocyte
OHSS	Ovarian Hyper-stimulation Syndrome
OPU	Ovum Pickup
OR	Ovarian Reserve
OS	Ovarian Stimulation
PGD	Preimplantation Genetic Diagnosis
PGS	Preimplantation Genetic Screening
rFSH	Recombinant Follicle Stimulating Hormone
UON	University of Nairobi

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ABSTRACT.

Background: Treatment of infertility is a major global problem which has remained a challenge in many ways. The cornerstone of in vitro fertilization (IVF), which forms the end point in care of infertile couples, is controlled ovarian stimulation (COS). However, predictability of outcomes has remained elusive despite identification of several biomarkers. Metaphase II (MII) oocytes are the mandatory prerequisite to IVF. However, there appears to be no relevant studies that focus on prediction of MII outcomes as an indicator of the potential for fertilization in patients that are undergoing IVF. This study focuses on the value of serum AMH in predicting MII oocyte outcomes in women undergoing COS.

Objective: To determine the role of serum AMH as a predictor of Metaphase II (MII) oocyte yield during controlled ovarian stimulation in a private fertility clinic in Nairobi.

Methodology: Retrospective descriptive cohort study that compared the processes in ovarian stimulation (OS) that culminate in production of MII oocytes in subjects with normal and low serum AMH. It was done at a private fertility clinic in Nairobi. Odds ratios (OR) and p values were used to compare the outcomes of OS between the two groups.

Results: Among those who had normal serum AMH levels, 17(28.2%) were aged more than 35 years as compared to19 (73.1%) among those who had low serum AMH (OR 0.1, 95%CI 0.1-0.4, p value <0.001). Normal response (5 to 14 follicles) on day 5 predominated in both groups but it was more preponderant among those with low AMH (normal AMH with 34 (57.6%) of the patients compared with 17 (65.4%) among those with low AMH, OR 0.7, CI 0.3-1.9, p<0.001). However, hyper response (≥15 follicles) occurred in 23 (39.0%) and 1 (3.8%) respectively (OR 15.9, CI 2.0-126.1, p<0.001) for normal and low serum AMH respectively. The pattern was similar on day 7 follicular count. Normal total oocyte harvest (5 - 14) occurred in 24 (40.7%) of patients with normal serum AMH compared to 9 (34.6%) among those with low AMH (OR 1.3, 95% CI 0.5 – 3.4, p value 0.597); while 24 (40.7%) and 1 (3.8%) of those with normal and low serum AMH respectively had ≥ 15 oocytes (OR 17.0, 95% CI 2.2 – 135.2, p value <0.001). There were 19 (73.1%) of patients with low serum AMH who had low MII oocyte yield compared to 16 (27.1%) in patients with normal serum AMH (OR 0.3, 95% CI 0.1-0.8, p value 0.014) and this difference persisted after controlling for age. Among those with normal serum AMH, 30 (50.8%) had MII oocyte yield between 5 to 14 oocytes compared to 7 (26.9%) of patients with low serum AMH

(OR 2.8, 95% CI 1.0-7.7, p value 0.040) whereas 13 (22.1%) of patients with normal serum AMH had MII oocyte yield of more than 15 oocytes compared to none in patients with low serum AMH. The sensitivity, specificity, positive and negative predictive values of serum AMH as a predictor of MII oocyte yield were 86.0%, 54.3%,72.96% and 73.1% respectively. The trend was similar for total oocyte harvest.

Conclusion*:* Serum AMH is a qualitative and quantitative predictor of MII oocyte yield as well as the preceding total oocyte harvest and follicular count. Hence, the levels of serum AMH can be used to provide counselling on possible outcomes of COS.

Key words: *Prediction with Serum AMH, follicular count, total follicular harvest, metaphase II oocyte yield.*

CHAPTER 1: INTRODUCTION

Ovarian stimulation (OS) is the process of inducing ovarian follicular development and oocyte maturation using medication[1]. The ultimate objective of OS is to achieve a pregnant state.

Infertility is a global health problem on average affecting approximately 20% of couples worldwide[2,3]. Assisted reproductive technology (ART) is the pinnacle of care where all other methods are not possible. However, there is preponderance of limitation in adequacy of treatment either because of regulatory frameworks and government policies that limit number of cycles and embryo transfers in developed countries and absolute lack of skilled manpower and assisted reproductive technology (ART) facilities in a majority of the developing world and hence limiting the beneficiaries of ART technology[4,5]. Socio-economic factors relating to cost of training and of the services have remained deterrents to advanced care of infertility through ART[4]. In developed countries, most of them may have free services but there is limitation of the number of embryos transferred and number of cycles[4]. Worse still, is lack of regulatory frameworks in many countries, lack of skills and the government commitment to infertility care [4]. In relation to COS, a key step is the ability to predict oocyte outcomes as a mandatory prerequisite to IVF. As a whole, poor success rates coupled with cancelled cycles limit the success rates[6]. Worse still is the limitation of the ability to predict oocyte quality outcomes, metaphase II (MII) oocytes in addition to fertilization and pregnancy rates and the eventual live birth rates (LBRs)[7,8,.9.10]. However, despite the limitations of biomarkers and particularly Anti – Mullerian Hormone (AMH) and antral follicular count (AFC), there is a correlation with ovarian reserve and number of oocytes retrieved including fertilization rates[9,11,12,13,14,15]. The fertilizable oocytes are those in Metaphase II (MII) and the real prediction should focus on the MII counts and proportions which are not influenced by sperm quality as is the case in fertilization rates and therefore, this would constitute the real potential in prediction of a likelihood of a positive outcome. This study focusses on the MII oocyte outcomes in relation to serum AMH levels.

CHAPTER 2: LITERATURE REVIEW

One of the greatest disasters in reproductive health is loss of capacity to reproduce in a world where universal participation in perpetuation of species is nearly universal. This loss of capacity to reproduce is experienced by up to 10 - 20% of couples in the global population[2,3], generally the prevalence being higher in developing countries[16,17]. Ovulation is a common pathway in reproduction and a certain proportion of infertile women will require ovulation induction as the primary treatment while a significantly large proportion would require COS for the process of IVF. According to Gerais et al, the cause of infertility in up to 66% of women in Africa is tubal factor[18]**.** The implication of this observation is that potentially, a majority of the women with tubal infertility would require COS for IVF.

Oogenesis and folliculogenesis

Females are born with the potential to reproduce, which begins in utero at the fifth week[19]. The process of oogenesis by meiosis starts in utero but gets arrested at diplonema stage of prophase I[20]. After puberty, cohorts of oocytes are recruited periodically and sequentially to undergo the process of folliculogenesis and get arrested at metaphase II (MII), which is the mature and fertilizable oocyte[20]. Fertilization re-initiates the process of completion of meiosis[20,21]. In a natural ovarian cycle, the process of recruitment of oocyte cohorts take place through reactivation of the process of meiosis at and after puberty but often only the dominant follicle matures[20]. On the other hand, in stimulated cycles for IVF, the target is to achieve multi-follicular development[22,23]. However, the process of ovulation is compounded by the reduction of ovulatory capacity due to precipitous decline in ovarian reserve (OR) with age above thirty five years [24,25]. This age-related reduction in oocyte competence is, in part, attributable to oxidative stress and mutations in the mitochondrial genome which is independent from the ovarian reserve[26,27]. The struggle to achieve the ability of participating in the perpetuation of the human species started from time immemorial but the greatest breakthrough was the development of the ability to stimulate ovarian function in order to get multiple oocytes for fertilization[22,23]. Thus, the primary objective in COS for natural fertilization is to mimic the natural process of monofollicular development, while in IVF, to

eliminate follicular dominance in order to achieve multi-follicular development and harvest many Metaphase II oocytes.

Historical perspective on ovulogens

Development of ovulogens

One of the greatest developments was the discovery of the ability to stimulate follicular development which is referred to as ovulation induction initially targeting anovulatory women[28]. The first report of successful ovulation induction using pituitary gonadotrophins was by Gemzell in 1948; whose work was confirmed by Buxton and his colleagues in Yale University in 1960[28]. This discovery was met by challenges requiring pituitary extracts from many individuals, lack of standardized dosages of gonadotrophins and risk of transmission of Jacob Creutzfeld disease (which was lethal in some cases) [28]. Another key development occurred when Donini discovered the technique of extraction of urinary human menopausal gonadotrophin from postmenopausal women which though was met with challenges of impurities, was able to have multi-follicular development resulting in multiple pregnancies[28]. However, standardization of the dose of gonadotropins and hypersensitivity reactions remained a challenge[28]. The development of Metrodin, a more purified urinary gonadotrophin with minimal luteinizing hormone by Serono, led to better standardization of dosages which was more effective in anovulatory cycles and later superovulation[28]. The synthesis of human recombinant follicle stimulating hormone (rFSH) using Chinese hamster cell lines by transfection resulted in unlimited production of this gonadotrophin, ushering in a new era of ovarian stimulation[29]. Currently, recombinant human follicle stimulating hormone from Chinese hamster ovary cells can be produced in serum free medium providing the advantages of both reducing risk of transmission of infection to humans and maintaining the efficacy of stimulation of folliculogenesis when used in OS similar to rFSH produced in serum[30]. More recent advancements have led to the development of the first human cell line - derived FSH called follitropin delta[31]. The recombinant technology has provided an advantage of good dosage control and hence individualised controlled ovarian stimulation (iCOS)[31]. Recombinant FSH was pure with the same sequencing of the pituitary FSH and allowed proper standardization of the administrable dosages. This ushered in the new era of standardization of ovarian stimulation. However, many issues on OS remain pending and subsequent outcomes such as continuing pregnancy rates (CPRs) and live birth rates (LBRs) still remain a challenge and in particular

prediction of MII oocyte outcomes does not appear to be significantly considered in OS evolvement and development.

Stimulation protocols

The objective in the use of stimulation protocols is optimizing stimulation outcomes in terms of oocyte harvest without compromising the safety of the patient particularly ovarian hyper-stimulation syndrome (OHSS). The objective in achieving adequate numbers is in the hope that many MII oocytes would be harvested but predictability remains low. In order to optimize follicular growth, the response to gonadotrophins is monitored on day 5 and day 7 routinely in all patients. This allows adjustment of the dosages of gonadotrophins in order to optimize follicular growth outcomes.

The agonist protocol achieves complete downregulation of the pituitary and therefore the ovary is fully at the disposal of exogenous simulation. On the other hand, the antagonist protocol involves initial start off with stimulation without down regulation and then partial down regulation in the mid-cycle in order to prevent early LH surge. Thus, the agonist protocol tends to yield more oocytes than the antagonist protocol and the latter principle is used in women perceived to be at risk of OHSS[32]. Literature review does not reveal that either of the protocols is associated specifically with a greater proportion of MII oocyte yield.

One of the markers of success in ovarian stimulation would be the ability to produce adequate good quality MII oocytes in preparation for in vitro fertilization. However, this consideration does not appear to be emphasized or feature in research and literature review. The main objectives in ovarian stimulation are safety in achieving multi-follicular development and to achieve adequate endometrial thickness through endogenous oestrogen stimulation[33]. The recent trend is towards use of biomarkers such as AMH, AFC, estradiol in order to individualize ovarian stimulation, targeting better OS outcomes [31,34,35]. Despite this, the overall global problem is deficiency in protocol standardization. This is in spite of continual search for protocols with different service providers adopting their own protocols without standardization[35].

Predictive use of ovarian biomarkers

The value of biomarkers in ovarian stimulation is in no doubt despite lack of uniformity in response probably because of confounders[35,36]. AMH, which is produced by preantral and early antral follicles, is currently considered as the most important biomarker in terms of predictive value and offers the advantage of low intercycle variability[7,10,34,35]. Bosch et al has demonstrated that AMH is currently leading in prediction of ovarian response and is superior to AFC, follicle stimulating hormone (FSH) and age in prediction of ovarian response, although in individualization probably a combination of multiple biomarkers should be used in prediction of ovarian response[34,35].

Laboratory

Discovery of use of transvaginal scan in retrieval has improved safety and oocyte yield significantly. Embryo grading in order to identify MII oocytes has also improved outcomes[37]. Similarly, landmark developments include refinement in embryo culture which now is often extended to day five (5) in order to transfer blastocysts. Since MII oocytes are the fertilizable oocytes, it is crucial that ways of prediction of MII oocyte yield be searched for.

Challenges in ovarian stimulation

Better understanding of OS opened doors towards the possibility of multi-follicular development which improved success rates as opposed to mono-follicular development in natural ovarian cycles as was the case with the conception of Louise Brown[22,24]. However, this happiness was short-lived because of the challenges of OHSS on one hand and poor response on the other[23,28,32,35,38,39]. Despite the advantages accrued through advancement of recombinant technology and research in OS, predictability of OHSS, a potentially lethal complication is low[32]. This is because reliable accurate parameters for prediction of ovarian response have remained a major challenge[32]. However, various strategies have been developed to minimize the risk of developing OHSS. These include: use of GnRH antagonists which yields fewer oocytes, coasting, avoiding use of human chorionic gonadotropin (HCG) for the trigger of oocyte maturation before retrieval in favour of agonist trigger, elective cryopreservation of all embryos and cycle cancellation in those patients perceived to be at very high risk[32]. The challenges in prediction, diagnosis, monitoring of OHSS and its management prevails up to today[32]. Part of the evolution of management includes the use of transvaginal scanning for ovum pick up (OPU)[24,40]. This, coupled with the great strides in the

evolution of IVF laboratories, resulted in much increase in the safety of IVF and improved success rates[24,41]. The key steps have been more objectivity in embryo grading and where possible, preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD)[24]. However, in most instances only morphologic classification is applied in IVF[37]. Although AMH is used as a predictor of hyper response and poor response, the outcomes are not consistent even after control of other factors such as age specifically lacking is predictability of MII oocyte outcomes, the mandatory fertilizable outcome in OS[10,42,43]. Overall, it is inferable that predictors of OS outcomes remain by and large inadequate and, specifically, the description of predictors of MII oocyte outcomes are virtually lacking.

From a global perspective, treatment of infertility remains a major challenge and the intricacies of ovulation stimulation and control remain significantly enigmatic[5,44]. This is in spite of the rapid growth in this field, including development of intracytoplasmic sperm injection (ICSI), embryo culture, vitrification and embryo transfer techniques[24,40]. Unspoken and by and large missing in the literature is the proportion of MII oocytes as outcomes in stimulated cycles. MII oocytes are the ultimate good quality oocytes based on morphologic classification of oocytes and the mandatory prerequisite to ICSI – anticipation of which should be the priority outcome. Therefore, this constitutes a major gap in prediction of success rates in stimulated cycles.

The value of optimizing the number of harvested oocytes is not in doubt. The classic study by Allegra et al has shown that the number of oocytes harvested is proportionate to the continuing pregnancies and that individualization of ovarian stimulation is key particularly using the normogram in order to determine the appropriate initial FSH dose[34]. The other studies also support the value of iCOS[23,31,35]. The challenge however remains that the normogram has not been shown to be universally useable. These studies however, fail to provide information on predictability of MII oocyte outcomes despite being the mandatory fertilizable oocyte outcome.

This study is designed to find credence in the value of AMH in prediction of MII oocyte outcomes as the mandatory necessity in IVF.

JUSTIFICATION:

MII oocyte is an obligatory prerequisite to ICSI and therefore, fertilization rates are highly dependent on their numbers in any OS cycle. Hence, the absolute number and proportion of MII oocytes are important in achieving fertilization and the number of cleaving grade I embryos. Unlike fertilization rates, the outcomes of MII oocytes are not related to sperm quality, a factor which is eliminated in the study. In IVF, there is a risk of hyper response and poor response which both influence cancellation of the cycle. However, it is not known even with hyper response and minimal response whether AMH predicts the likelihood of MII oocytes. This is therefore irrespective of whether there is quantitative prediction or not. A comparison of predictive values of normal and low biomarkers gives or rules out credence of their use in prediction of effective response in achieving the desired quantity and quality of oocytes. Currently assisted reproductive services (ART) in Kenya and in Africa are at its infancy and there are no regulatory frameworks. This puts the practice at disarray without adequate legislative and policy guidelines. Therefore, MII oocyte outcome provides the real exploitable fertilization potential in classical IVF and ICSI, creating a need for this understanding which is translatable into policy and clinical practice.

CONCEPTUAL FRAMEWORK

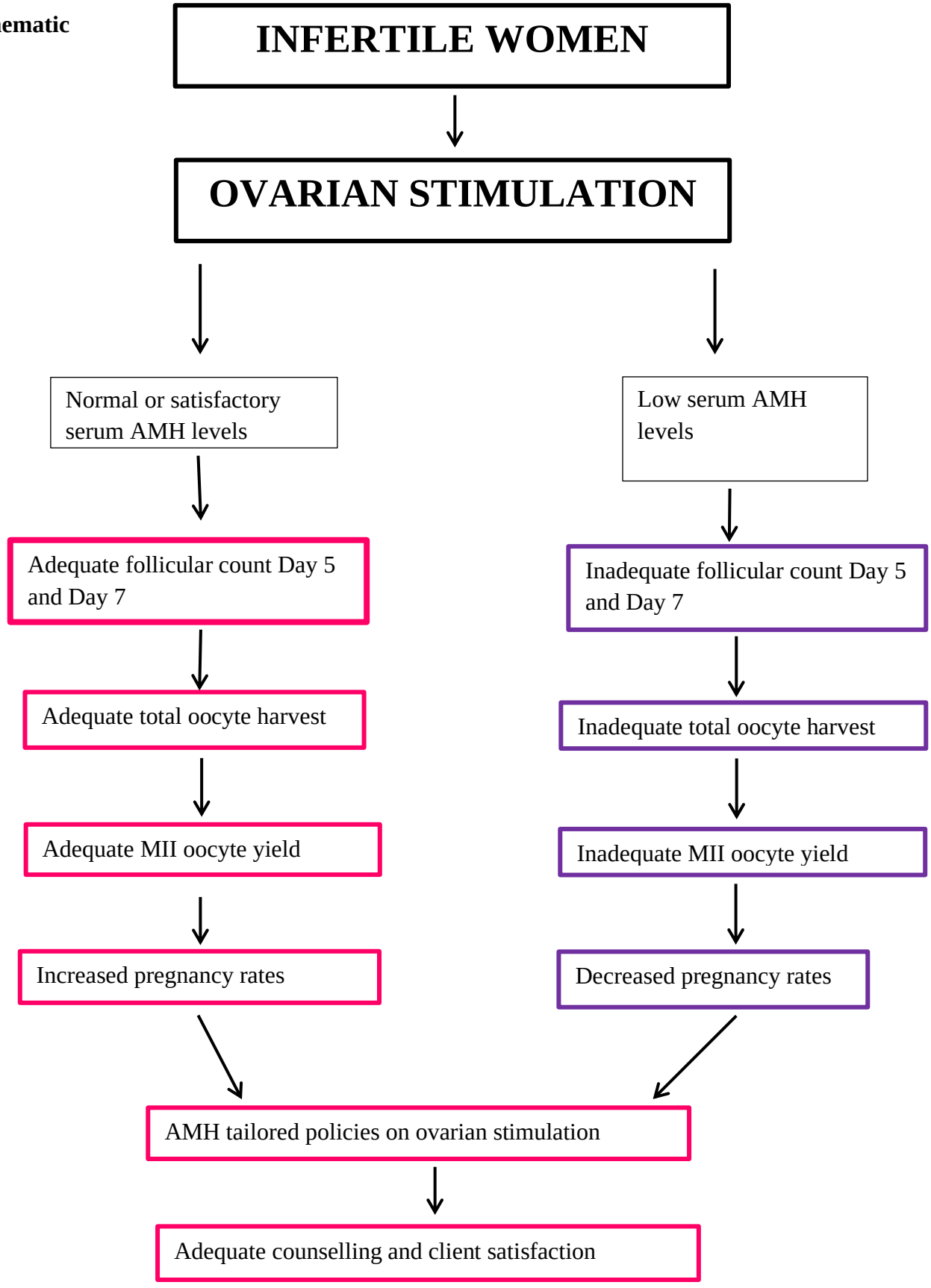
i) Narrative

AMH significantly predicts ovarian response which is measurable in terms of follicular count from day 5 intermittently. Day 5 and day 7 total follicular counts are used as the intermediate outcomes upon which dosages are adjusted in individualized controlled ovarian stimulation. Total oocyte yield and specifically MII oocyte yield as the ultimate indicator of potential for the occurrence of pregnancy. Important correlates are age and serum AMH levels. It is expected that follicular count correlates well with the serum AMH levels. However, what potentiates pregnancy rates is the proportion of MII oocytes. Normal serum AMH levels will be expected to yield adequate oocytes while low AMH may be expected to yield less MII oocytes. But this area has not yet been exploited. Although there has been relatively wide and growing acceptance of using AMH as a biomarker for prediction of ovarian response, guidelines and policies on its use have not been developed and research towards efficacy is still wanting. If serum AMH is shown to be an effective predictor of fertilizable oocytes (MII oocytes) as an outcome of COS, this would be translatable into better individualization of COS (iCOS) – with less cycle cancelation rates due to OHSS or

poor response, better outcomes of OS and the benefit would be cascaded into increased LBRs. In addition, there will be better counselling and psychological preparedness of the patients. Policies on OS are also likely to evolve from such outcomes.

This finding is important because it would help in stimulation decision making and providing pre-stimulation counselling and outcomes which is the anxiety of both the service provider and the patients. In addition, this will help formulate policies on OS based on expected outcome.

ii) Schematic



RESEARCH QUESTION:

Is serum anti-mullerian hormone level a predictor of Metaphase II oocyte yield in controlled ovarian stimulation?

HYPOTHESES:

Null hypothesis (Ho): Serum AMH level is not a predictor of Metaphase II oocyte yield in controlled ovarian stimulation.

Alternate hypothesis (H_1): Serum AMH level is a predictor of Metaphase II oocyte yield in controlled ovarian stimulation.

OBJECTIVES:

Broad Objective:

To determine the role of serum AMH as a predictor of Metaphase II oocyte yield during controlled ovarian stimulation in a private fertility clinic in Nairobi, 2013 to 2019.

Specific Objectives:

In order to determine the role of serum AMH as a predictor of Metaphase II oocyte yield during controlled ovarian stimulation in a private fertility clinic in Nairobi, 2013 to 2019 the following was compared:

1. Intracycle follicular count in day five (5) and day seven (7) by levels of serum AMH.
2. Total follicular harvest by levels of serum AMH.
3. Metaphase II (MII) oocyte yield by levels of serum AMH.
4. Sensitivity, specificity, and predictive value of serum AMH levels in predicting MII oocyte outcomes.

CHAPTER THREE: METHODOLOGY

Study site:

The study site was at the Nairobi Fertility Clinic in Professor Nelson Awori Centre which is adjacent to Nairobi Hospital along Ralph Bunche Road. It is a private clinic that recruits patients with infertility and others are referred from neighbouring clinics and beyond for IVF. Follow up after treatment is done at the source of the patients. Acute complications such as OHSS are managed in collaboration with the ART specialists in the clinic. The clinic is approximately 1 kilometre from the School of Medicine, College of Health Sciences, University of Nairobi, Kenyatta National Hospital campus. The clinic provides comprehensive ART services to couples suffering from primary or secondary infertility. The clinic is ideal for this study as it serves couples from similar socioeconomic status, is one of the few established IVF centres in Nairobi and Kenya at large, with a consistent adequate number of patients who can afford this expensive service. A batch approach is used in ovarian stimulation every quarter using combined oral contraceptives for synchronization of the menstrual cycles. The advantages of using Nairobi Fertility Clinic data in this study were the quality and completeness of the records that are kept, and the ease of retrieval of serum AMH level results from Medipath Laboratories database. For these reasons, the study site was ideal for this study.

Study design:

The study design was a retrospective descriptive cohort of women who had undergone COS within a period of six years. The actual duration of the study within this period was dependent on the achievement of the desirable sample size based on the fulfilment of the inclusion criteria. The study also exploited the milestones of the processes of OS, which ultimately culminates in oocyte harvest. The choice of retrospective cohort in a situation as this where records are very good is deemed appropriate as data collection has occurred in a natural environment and hence, bias is minimal. Patients with at least prediction of satisfactory and optimum results of serum AMH (1.00 to 3.99 ng/mL and 4.00 to 6.80 ng/mL respectively) consisted one group of the study while those with predicted low response levels of serum AMH (0.20 to 0.99 ng/mL) constituted the other group in the cohort. All files from the last batch downwards up to the previous six years were studied. The relevant data was extracted from each category based on the AMH levels using a specific assay (VIDAS®) for uniformity of interpretation until the sample size was reached in

each group. In the occasional event when there was deficiency of vital data in either of the groups, this was considered as a non-response, which occurred at random and hence was assumed not to have any significant influence on desired outcomes.

Study population:

The study population consisted of women with primary or secondary infertility who had undergone OS for IVF and met the inclusion criteria. These women underwent OS using the long agonist protocol. In all instances pituitary downregulation should have been through use of long-acting gonadotrophin releasing hormone (GnRH) analogue. All of them had cycle synchronization using combined oral contraceptives (COC). The records of pre-stimulation general characteristics data were extracted from the files and matched with stimulation outcomes up to two hours after ovum retrieval by which time categorization of MII oocyte yield was completed in time for ICSI. The two hours wait after oocyte denudation allows conversion of more Metaphase I oocytes (MI) to Metaphase II oocytes (MII) and therefore optimizing MII oocyte yield. Groups in the cohort consisted of women who had serum AMH values between 1.00 to 6.80 ng/Ml while group B consisted of women between 0.20 ng/mL to 0.99 ng/mL.

Study instrument:

The data collection instrument was a questionnaire or checklist based on the objectives:

Serum AMH levels were recorded together with the serial number of the questionnaire since it was used for categorization. Group A were those with favourable AMH in prediction of response while group B were those with prediction of low response. Therefore, the AMH grouping and the serial number were the nomenclature of the categorization of the two groups.

The questionnaire had the following sections based on the objectives:

SECTION A: General characteristics such as age, serum AMH levels, primary type of infertility, secondary type of infertility and causative factor of infertility.

SECTION B: Serum AMH values and categorization of predicted ovarian response designated as satisfactory or optimum in one group and low response in the other group.

SECTION C*:* Follicular count on day five (5) and seven (7).

SECTION D: Total oocyte harvest and MII oocyte yield. (< 5 oocytes low response, 5-14 normal response, >15 hyper response)

Inclusion criteria:

1. Women who have been evaluated and diagnosed with primary or secondary infertility.
2. All women who are 18 years and over and below the age of 45 years.
3. Women who were having their first stimulation cycle in the clinic.
4. Ovum donors that underwent controlled ovarian stimulation with long agonist protocol.
5. Women who have completed stimulation and have had oocyte retrieval.
6. AMH values were assayed at Medipath Laboratories using the VIDAS® kit in ng/mL.
7. Women who underwent controlled ovarian stimulation with the long agonist protocol.

Exclusion criteria:

1. Women under 18 years and above 45 years.
2. Women who have concurrent endocrine disorders such as hyperprolactinemia and thyroid disease which are known to deter ovarian response independently.
3. Women subjected to GnRH antagonist protocol or ultra-short agonist protocol.
4. Patients who had high serum AMH values beyond 6.80 ng/mL were not included in the study due to increased risk of OHSS.

Data collection

The data was collected sequentially from the clinic records without using any special sampling procedure. Categorization was serial through selecting the records that fitted the inclusion criteria. On one hand was the group that had serum AMH levels from 1.00 ng/mL to 6.80 ng/mL, whose response was predicted to be good; on the other hand were women with serum AMH levels from 0.20 ng/mL to 0.99 ng/mL whose response was predicted to be low.

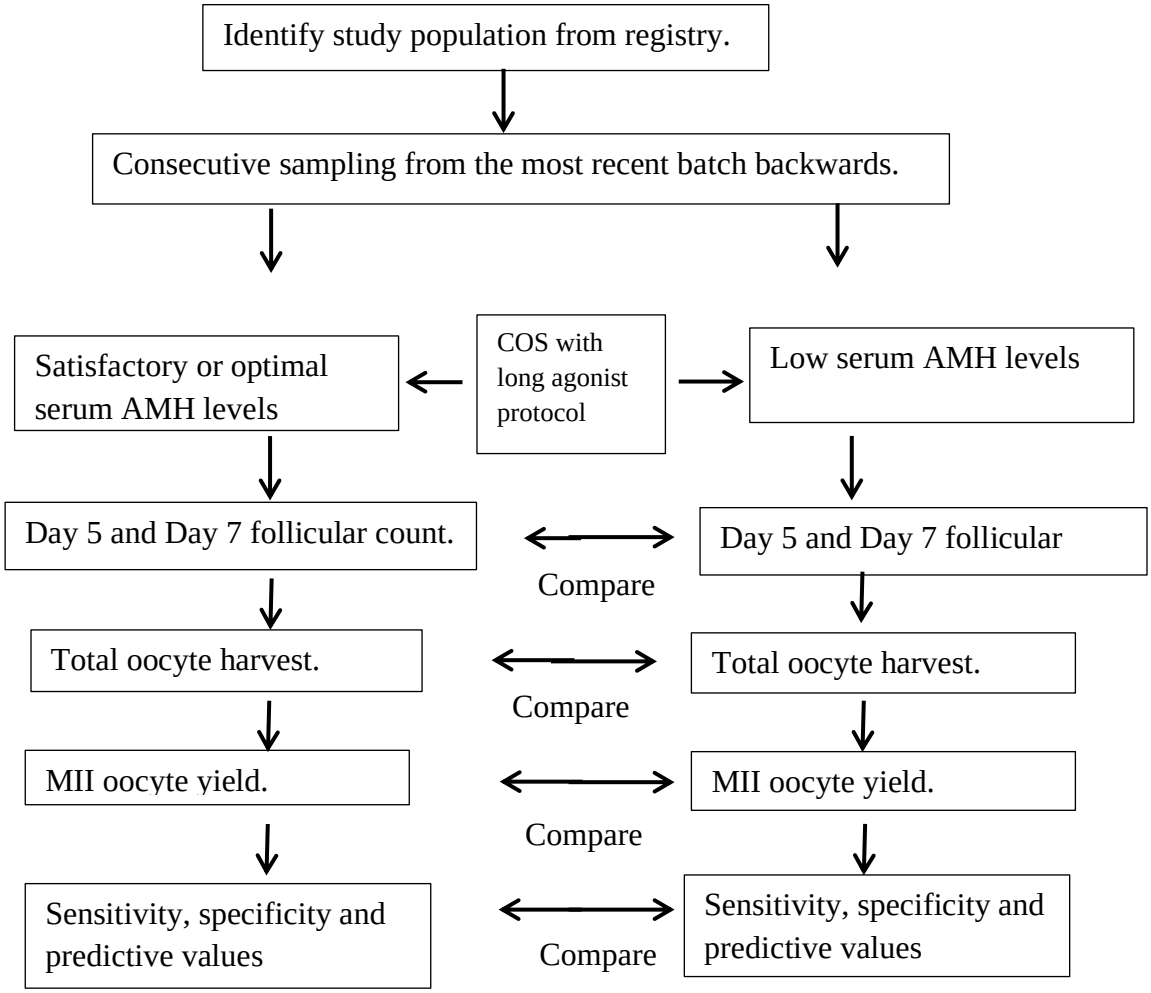
The process of selecting the study population was done through sequential review of results and their records extracted from the register at the clinic based on the inclusion criteria. Scrutiny was done depending on the levels of serum AMH to allot the subject to either of the groups. This process continued until each of the groups sequentially attained the desired sample size.

The serum AMH assay was done using the VIDAS® AMH (AMH) using the Enzyme Linked Fluorescent Assay Technique. In a study done by Pastuszek E et al comparing the newer VIDAS® AMH assay kit with the Elecysis® shows similar values thereby validating the assays done using

VIDAS® kit[45]. The nomenclature was in ng/mL. The data was collected sequentially until the desired sample size was arrived at a ratio of 1:1 for both study populations. Data collection started from the most recent batch backwards. Data was extracted from the records and transferred to the data collecting instrument. It was estimated that data collection would take three (3) months. The approach in data collection involved sequential identification of the patient's names and then retrieval of the specific laboratory results only from Medipath Laboratories. This prevented intra and inter observer errors. Any data that was missing was considered as non-response. This was not expected to introduce any bias as it was assumed to be occurring at random.

Prior to data collection, the principal investigator obtained permission from the Clinic Directors and liaised with the clinic manager in order to access records. The names from each batch were obtained from the clinic register, which were then used to retrieve serum AMH levels from the clinic files or from Medipath Laboratories records. The files were scrutinized sequentially and the relevant data obtainable transferred to the data collecting instrument. The COS outcome data was retrieved from the clinic computer and matched with the hard copy data in the data collection instrument.

Data collection flow chart



Sample size

Since there were no relevant similar studies that could be obtained from extensive literature search two (2) assumptions were made for this retrospective cohort study. For the first group which is supposed to demonstrate higher MII oocyte yield 50% was presumed to be the yield rate. For the second group with low AMH and lower anticipation of MII oocyte yield, a presumption of 25% of MII oocyte yield was made. Based on the formula by Kelsel et al, the sample size was calculated as follows:

$$n_1 = \frac{(Z_{\frac{\alpha}{2}} + Z_{1-\beta})^2 \bar{p}\bar{q}(r+1)}{r(p_1 - p_2)^2}$$

and

$$n_2 = rn_1$$

Where;

n_1 = Patients in group A; least prediction of satisfactory results (to be estimated);

n_2 = Patients in group B; with low response (to be estimated);

$Z_{\alpha/2}$ = Standard normal deviate for two-tailed test based on alpha level (set at 5%).

$Z_{\beta/2}$ = Power of the study based on beta level (set at 80%).

r = ratio of group B to group A (set at 1:1).

p_1 = proportions of group A success/response rate (estimated at 50%).

p_2 = proportions of group B success/response rate (estimated at 25%).

$\bar{p} = (p_1+rp_2)/(r+1)$ and

$\bar{q}=1-\bar{p}$

Substituting the value into the equation above we get;

$$\begin{aligned} n_1 &= \frac{(1.96 + 0.842)^2 \times 0.375 \times 0.625 \times (1+1)}{1(0.5 - 0.25)^2} \\ &= \frac{2.802^2 \times 0.234 \times 2}{0.25^2} \\ &= 58.5 \\ &= 59 \end{aligned}$$

$$\begin{aligned} n_2 &= rn_1 \\ &= 59 \end{aligned}$$

(*Reference used: Kelsey JL, Whittemore AS, Evans AS, Thompson WD. Methods in Observational Epidemiology. Oxford University Press, 1996.)*

The calculated sample size was 59 patients in each group which may be increased depending on the number of patients available in order to increase the power of the study and allow cross tabulations during analysis.

Sampling procedure

Consecutive data collection on women fulfilling the inclusion criteria without any special sampling techniques until the desired sample size was reached for each group.

Recruitment and consenting procedure

Since this was retrospective data, this was not necessary as all data was retrieved from existing files. The consent to use the files was obtained from the Clinic Directors.

Data collection procedures

Data collection site was in the fertility clinic and no raw data, files or results bearing names and identification numbers of the patients were moved out of the record area.

Study variables

The variables in this study were divided into independent variables and dependent variables. The independent variables were:

1. The general characteristics of women suffering from infertility.
2. Serum AMH.

The dependent variables were:

1. Intra-cycle follicular count in day five (5) and day seven (7).
2. Total follicular harvest.
3. Metaphase II (MII) oocyte yield.

Day five (5) and day seven (7) intra-cycle follicular counts were chosen as indicators of response because they are universally recorded as a key aspect of effecting individualization of COS in this IVF clinic. They are therefore important milestones of adequacy of response during COS.

In this study, low response was taken as less than 5 oocytes, normal response was taken as 5 to 14 oocytes and hyper response was taken as 15 oocytes and above. In Nairobi fertility clinic, cycle cancellation for hyper response is usually considered when the follicular count on day 7 is 30

follicles or more. Oocyte harvest refers to the number of oocytes retrieved irrespective of the follicles or the quality of the oocytes harvested. Total oocyte harvest is the actual yield of oocytes irrespective of quality. MII yield is the total of MII oocytes retrieved, which constitutes the fertilizable oocytes which therefore undergo intracytoplasmic sperm injection (ICSI).

Quality assurance procedures

Data collection was done by the principal investigator. All the data collected was scrutinized once again for correctness of entry. The study instrument strictly adhered to the study objectives. The data collected was not altered and was based on the records obtained without any alteration. Hence it was unlikely that bias would be introduced as the data was a replica of what was in the records.

Data analysis

Once data collection was complete, all answers to open ended questions were coded. Data was entered into the computer and cleaned, outliers identified and corrections made if any. Analysis was done using Statistical Package for Social Sciences (SPSS) version 24.0 and strata 15. Basic comparative frequencies were run for each study group, scrutinized and then grouped data analysis done in accordance to the study objectives. Final data was presented in comparative frequencies, cross-tabulations and diagrams as deemed necessary. Appropriate tests of significance were applied (Pierson, Chi-square and Student's T tests) where applicable, and a p value of <0.05 was considered statistically significant.

The sensitivity, specificity and predictive values were calculated using the formulae below based on the table in appendix III in the plan of analysis on page 46.

i. **Sensitivity** = $\frac{\mathbf{A}}{\mathbf{A+B}}$ %

ii. **Specificity** = $\frac{\mathbf{D}}{\mathbf{C+D}}$ %

iii. **Positive predictive value (PP)** = $\frac{\mathbf{A}}{\mathbf{A+C}}$ %

iv. **Negative predictive value (NPV)** = $\frac{\mathbf{D}}{\mathbf{B+D}}$ %

Study limitations

1. There was some missing information that was not vital to the study, which is expected in retrospective studies.
2. The sample size was not reached for the low serum AMH population since mixed kits had been used. This was however mitigated by cross tabulations which indicated that the effect persisted when controlled for age. In addition, statistical differences were seen between the patients with normal serum AMH and low serum AMH based on the p value and OR values seen. Since there were no previous similar studies of this nature, the sample size was calculated based on an assumption of 50% for the patients with normal serum AMH levels and 25% for patients with low serum AMH levels. Despite this limitation, the data was analyzable and there were statistical differences observed. This indicates that the calculated sample size was most likely an overestimate.

3. Although the sample size was adequate for analysis, a larger study with bigger sample size will allow alteration of design, data matching of controls, cross tabulations and elimination of confounders and stricter matching. Despite this the study did show a type II error indicative of the differences between the normal and low groups. The post hoc analysis showed the power at 58.2% which shows both scientific and statistical difference thus showing that serum AMH is indeed a predictor of day 5 and day 7 follicular count, total oocyte harvest and MII oocyte yield. This study is therefore a baseline study. Other studies can be conducted in future with larger sample size that will power the results and interpretation. The results of this study are therefore seen in this context. Therefore, there is need to do another study.
4. Currently, there are no universally standardized reference ranges of serum AMH on prediction of response as the anticipated effect is highly kit dependent.

Ethical considerations:

Clearance was obtained from the Department of Obstetrics and Gynaecology at UON, KNH-UoN ERC and directors from the clinic. All the information was kept confidential and the identity of the patients was de-identified. All the data was collected in the clinic as a measure to enhance confidentiality and no records were taken outside the clinic vicinity. Since this was essentially a clinical audit and data collection was retrospective, there was no contact with the patient, no informed consent was required

Conflict of interest:

There is no conflict of conflict of interest in the choice of Medipath Laboratories. The objective was to have a consistent and uniform assay and VIDAS® was deemed as one of the most reliable assay techniques.

CHAPTER FOUR: RESULTS, DISCUSSION, CONCLUSION.

RESULTS

There were 446 patients' files that were retrieved between November 2019 to May 2013.Of these, 59 files fulfilled the criteria for patients with normal AMH and 26 with low AMH, who were stimulated. These two categories form the basis for analysis in this study.

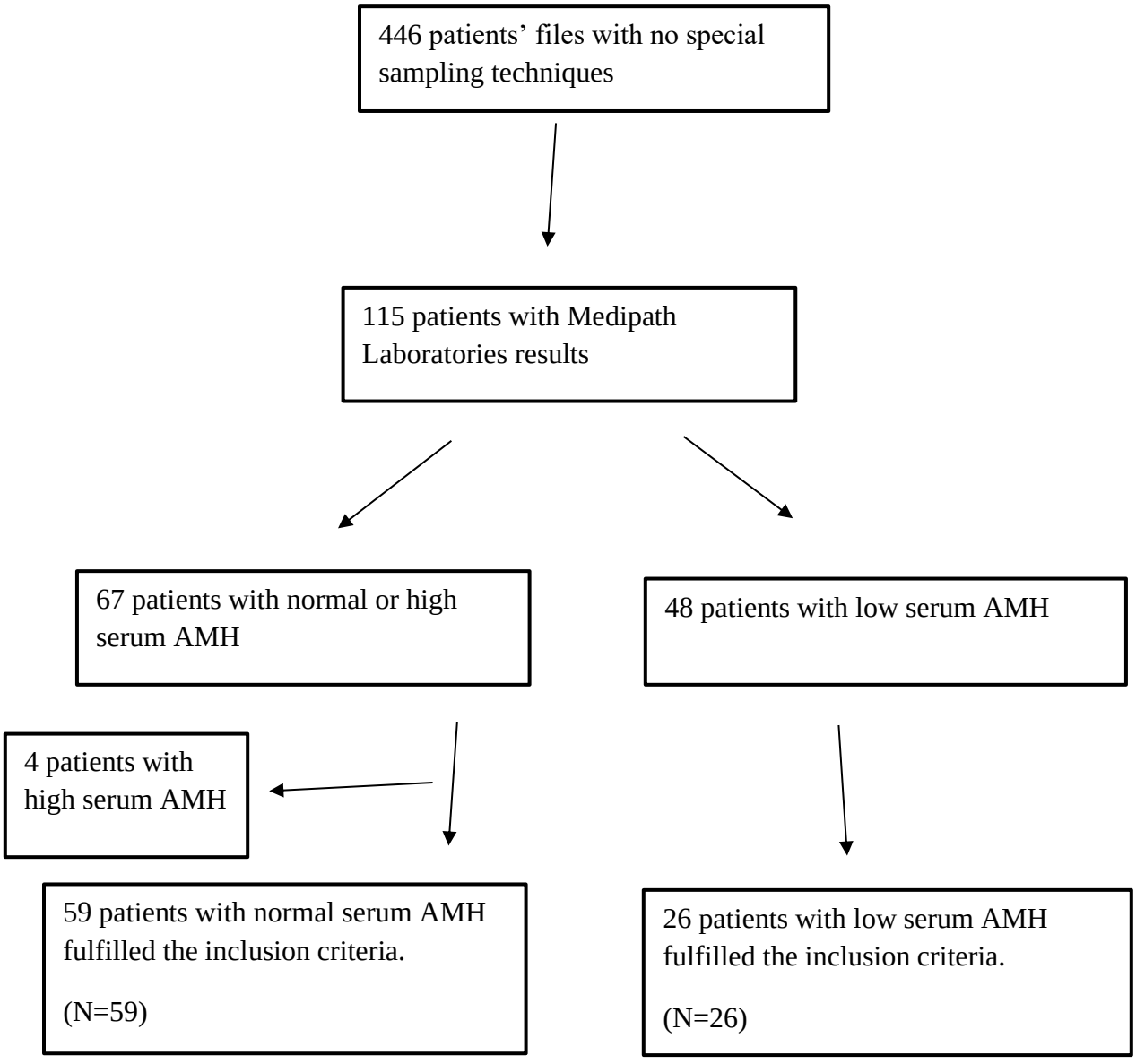


Figure 1: Diagrammatic representation of recruitment process.

Table 1. Selected general and reproductive characteristics of the study population by normal versus low serum AMH levels (N=85)

Characteristic	AMH level		OR (95%CI)	p value
	Normal (N=59) No. (%)	Low (N=26) No. (%)		
General				
Age (Completed yrs)				
<30	16 (27.1)	4 (15.4)	1.9 (0.5 -6.6)	0.274
30 – 35	26 (44.1)	3 (11.5)	6.3 (1.7 - 23.2)	0.003
>35	17 (28.8)	19 (73.1)	0.1 (0.1 - 0.4)	<0.001
Reproductive				
Type of infertility				
Primary	10 (16.9)	3 (11.5)	1.6 (0.4 – 6.2)	0.523
Secondary	10 (16.9)	7 (26.9)	0.5 (0.2 – 1.3)	0.150
Not indicated	33 (55.9)	16 (57.7)	0.8 (0.3 – 2.0)	0.629
N/A (Donors)	6 (10.2)	0	-	-
Cause of infertility				
Tubal factors	35 (59.2)	12 (46.2)	1.4 (0.6 – 3.6)	0.450
Ovarian factors	2 (3.4)	5(19.2)	0.1 (0.0 – 0.8)	0.013
Uterine factors	3 (5.1)	2 (7.7)	0.2 (0.0 – 2.3)	0.162
Male factors	10 (16.9)	7 (26.9)	0.8 (0.3 – 2.2)	0.596
Age factor	2 (3.4)	1 (3.8)	0.9 (0.1 – 9.9)	0.905
Unexplained infertility	11 (18.6)	5 (19.2)	1.3 (0.4 -4.0)	0.674
Previous stimulation cycles				
One	10 (16.9)	4(15.4)	1.1 (0.3 – 3.9)	0.882
Two	1 (1.7)	1 (3.8)	0.4 (0.0 – 7.0)	0.538
Three	-	-	-	-
>3	1 (1.7)	1 (3.8)	0.4 (0.0 – 7.0)	0.538
Nil	47 (79.7)	20 (76.9)	1.2 (0.4 – 3.6)	0.747

Table 1 shows that a majority of the patients 26(44.1%) with normal AMH were aged between 30 and 35 years inclusive compared to 3 (11.5%) of those who had low serum AMH (OR 6.3, 95%CI 1.7-23.2, p value 0.003) and this difference was statistically significant. Similarly, 17 (28.8%) of those with normal AMH were aged more than 35 years compared to 19 (73.1%) of those who had low serum AMH (OR 0.1, 95%CI 0.1-0.4, p value <0.001). There were no significant differences

in relation to the type of infertility, cause of infertility or history of previous stimulation among the two populations.

Table 2. Follicular count on day 5 and day 7 by normal versus low serum AMH levels (N=85)

Follicular count	AMH level		OR (95%CI)	p value
	Normal (N=59) No (%)	Low (N=26) No (%)		
Day 5				
Low response (<5)	2 (3.4)	8 (30.8)	0.1 (0.0-0.4)	<0.001
Normal response (5-14)	34 (57.6)	17 (65.4)	0.7 (0.3 – 1.9)	<0.001
Hyper response (≥15)	23 (39.0)	1 (3.8)	15.9 (2.0 -126.1)	<0.001
Day 7				
Low response (<5)	1 (1.7)	6 (23.1)	0.1 (0.0-0.5)	<0.001
Normal response (5-14)	22 (37.3)	19 (73.1)	0.1 (0.1-0.4)	<0.001
Hyper response (≥15)	36 (61.0)	1 (3.8)	39.1 (2.0-126.1)	<0.001

Table 2 shows follicular count on day 5 and day 7 by serum AMH levels. On day 5, normal response (5 to 14 follicles) predominated in both groups but it was more preponderant among those with low AMH (normal AMH with 34 (57.6%) of the patients compared with 17 (65.4%) among those with low AMH OR 0.7, 95%CI 0.3-1.9, p value <0.001) in favour of low AMH category. Hyper response occurred in 23 (39.0%) and 1 (3.8%) respectively (OR 15.9, 95%CI 2.0-126.1, p value <0.001). However, a combination of normal and hyper response constituted 57 (96.6%) of those with normal AMH compared to 18 (69.2%) of those with low AMH. On the whole, low response was more common among those with low AMH 8 (30.8%) than among those with normal AMH 2 (3.4%), OR 0.1, 95% CI 0.0-0.4, p value <0.001. Follicular count in day 7 showed similar trends and all these differences were statistically significant (p value<0.001 for all categories).

Table 3. Averages of follicular count on day 5 and day 7 by normal versus low serum AMH levels (N=85)

Average measure		AMH level		Student T test	p value
		Normal (N=59)	Low (N=26)		
Day 5					
	Mean	14.03	6.38	t = 4.1	<0.001
	Mode	7.00	6.00		
	Median	12.00	6.00		
	Range [Min-Max]	3 - 54 (51)	0 – 15 (15)		
	Range [IQR]	7 – 23	4 - 8		
	Standard deviation (2STD)	9.05	3.69		
Day 7					
	Mean	18.10	7.46	t = 4.2	<0.001
	Mode	15.00	6.00		
	Median	15.50	6.50		
	Range [Min-Max]	3 – 63 (60)	0 – 22 (22)		
	Range [IQR]	10-23	5-10		
	Standard deviation (2STD)	12.14	4.55		

The averages of follicular count by serum AMH levels are depicted in table 3. All the average indicators of follicular count on day 5 were higher among those with normal AMH as compared to those with low serum AMH. The mean follicular count on day 5 was 14.03 compared to 6.38 for the normal and low serum AMH groups respectively, where the median was 12.00 compared to 6.00 respectively. The range was very high 3 to 54 (51) compared to 0 to 15 (15) for the normal and low serum AMH categories respectively. The trend of these parameters was the same for day 7 with a much greater difference in the average indicators in favour of those with normal serum AMH. (It indicates that follicular count increases much further during stimulation than those with high AMH). The student t tests value was 4.1 and 4.2 respectively for days 5 and days 7 and the p value was <0.001 in both instances in favour of normal serum AMH. The trend was the same for the mode, median, range and standard deviation on day 7.

Table 4. Total oocyte harvest by normal versus low serum AMH level (N=85)

Oocyte harvest	**AMH level**		**OR (95%CI)**	**p value**
	Normal (N=59) No (%)	**Low (N=26) No (%)**		
Low response (<5)	11 (18.6)	16 (61.5)	0.1 (0.1 – 0.4)	<0.001
Normal response (5 – 14)	24 (40.7)	9 (34.6)	1.3 (0.5 – 3.4)	0.597
Hyper response (≥15)	24 (40.7)	1 (3.8)	17.0 (2.2 – 135.2)	<0.001

For total oocyte harvest, less than 5 follicles were predominant in the low serum AMH category compared to the normal serum AMH category 16 (61.5%) and 11 (18.6%) respectively, OR 0.1, 95% CI 0.1-0.4, p value <0.001). Those who had normal response (5 to 14 oocytes) were 24 (40.7%) and 9 (34.6%) in the normal serum AMH and low serum AMH categories respectively but this difference was not statistically significant (p value 0.597). However, those who had 15 oocytes and above were 24 (40.7%) and 1 (3.8%) for the normal serum AMH and low serum AMH categories respectively (OR 17.0, 95% CI 2.2-135.2, p value of <0.001). When normal and high oocyte harvest are combined, the proportion rises to 48 (81.4%) for the normal AMH category compared to 10 (38.4%) among the low AMH category, indicative of better response prediction with normal AMH. It shows hyper response thereby reducing the number of patients in the normal category creating a misnomer of apparent good response among those with low serum AMH.

Figure 2. Frequency distribution of total oocyte harvest by normal versus low serum AMH levels

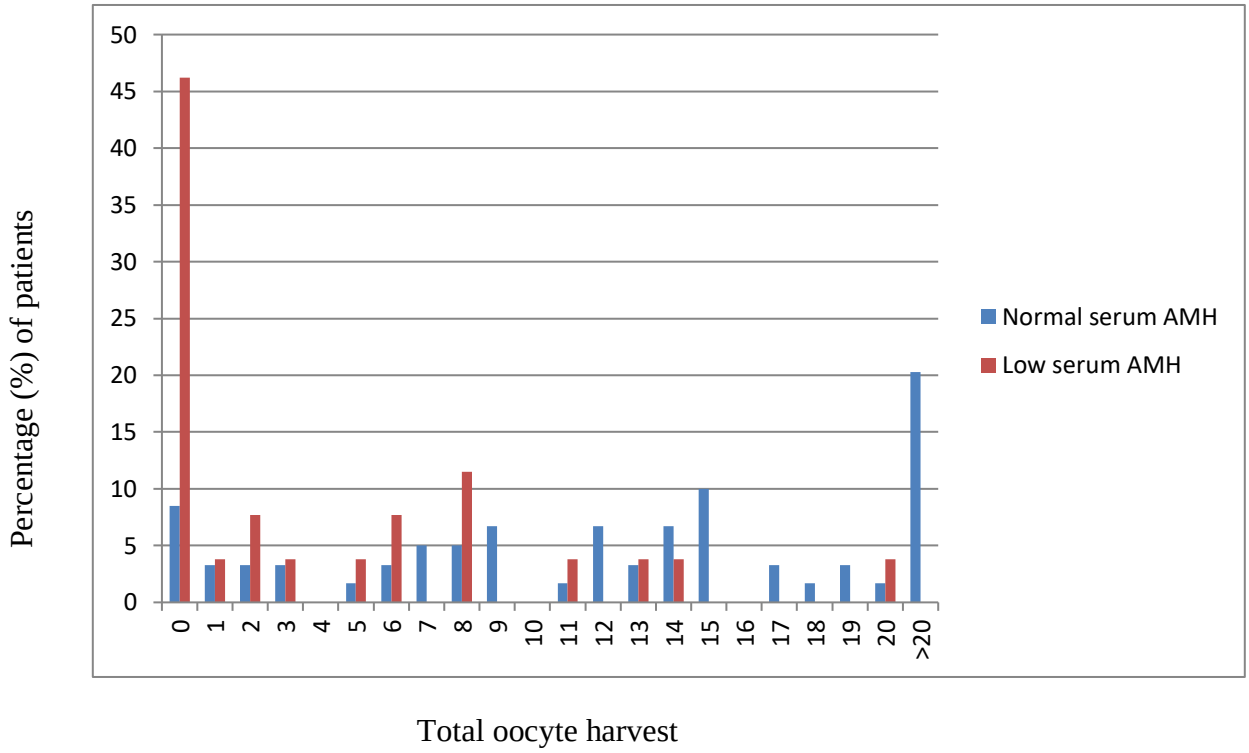


Figure 2 shows the frequency distribution of total oocyte harvest by serum AMH levels. Cancellation due to poor response were the most common constituting of 5 (8.5%) of subjects with normal AMH compared to 12 (46.2%) of patients with low serum AMH. Patients with more than 20 follicles constituted 12 (20.3%) of those who had normal serum AMH compared to none in patients who had low serum AMH. The frequency of higher response was more preponderant among those with normal AMH compared with those with low AMH and the risk of under response was higher with those with low AMH).

Table 5. Averages of total oocyte harvest by normal versus low serum AMH levels (N=85)

Average measure	AMH level		Student's t test	p value
	Normal (N=59)	Low(N=26)		
Mean	13.37	4.12	t=4.44	<0.001
Mode	15	0		
Median	13.0	1.50		
Range [Min-Max]	0-38 (38)	0-20 (20)		
Range [IQR]	7 – 19.0	0 – 8.0		
Standard deviation (2STD)	9.63	5.49		

The averages of total oocyte harvest, as depicted in table 6. were significantly higher for the normal serum AMH group compared to the low serum AMH group with the mean 13.37 and 4.12 respectively. The range for the normal and low serum AMH categories was 0-38 (38) and 0-20 (20) respectively and the standard deviation (2STD) 9.63 and 5.49 respectively. The differences between the two categories were statistically significant with a p value of <0.001, in favour of those with normal serum AMH.

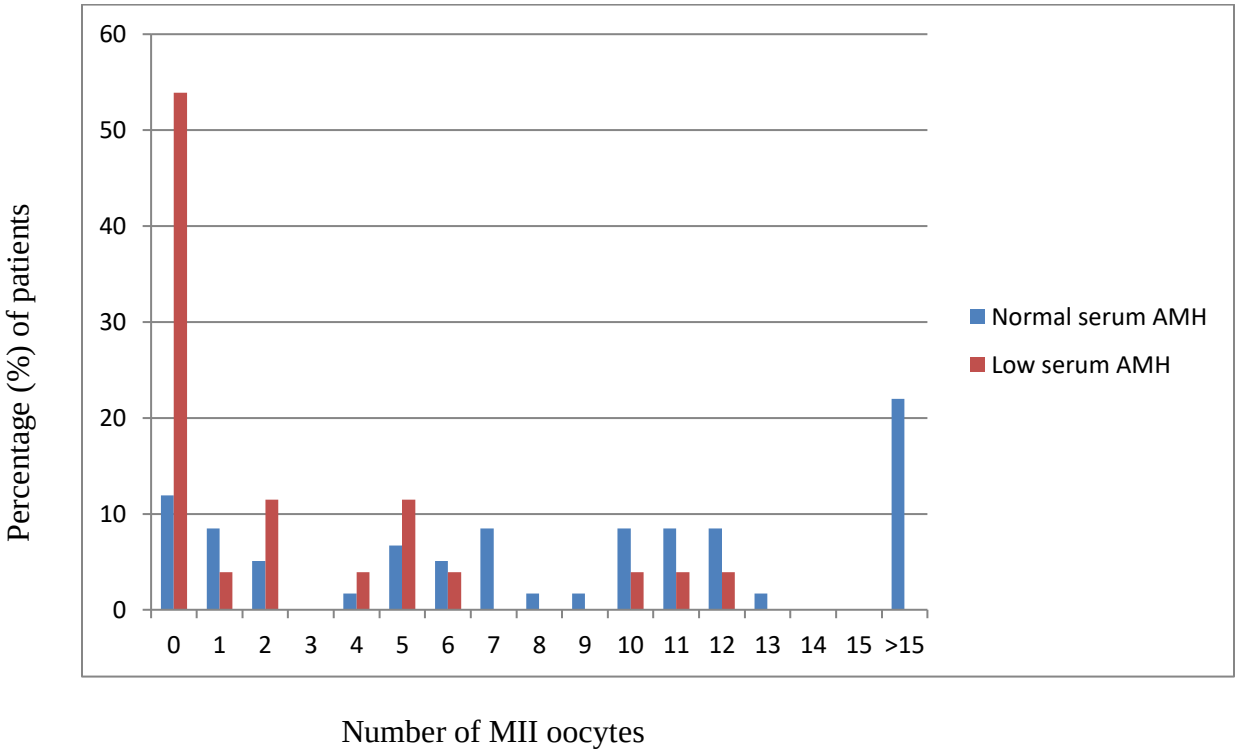
Table 6. Metaphase II (MII) oocyte yield by normal versus low serum AMH level (N=85)

MII oocyte yield	AMH Level		OR(95%CI)	p value
	Normal(N=59) No. (%)	Low(N=26) No. (%)		
<5	16 (27.1)	19(73.1)	0.3(0.1-0.8)	0.014
5-14	30 (50.8)	7 (26.9)	2.8(1.0-7.7)	0.040
≥ 15	13 (22.1)	-		

Table 6 shows MII oocyte yield by serum AMH level. Grouped data on MII oocyte yield by serum AMH category showed that 19 (73.1%) of the low serum AMH category had MII oocyte yield of less than 5 oocytes compared to 16 (27.1%) of the normal serum AMH category (OR 0.3, 95%CI 0.1-0.8, p value 0.014. MII oocyte yield of 5-14 was higher in the normal serum AMH group 30 (50.8%) compared to 7 (26.9%) in the low serum AMH group (OR 2.8, 95%CI 1.0-7.7, p value

0.040). MII oocyte yield of 15 or more was 13 (22.1%) in the normal serum AMH group compared to no yield in the low serum AMH group. These differences are statistically significant.

Figure 3. Frequency distribution of MII oocyte yield by normal versus low serum AMH level



As can be seen in figure 3, there was a preponderance of nil (0) MII oocyte yield in the low serum AMH group compared to the normal serum AMH group with 14 (53.9%) and 7 (11.9%) respectively (OR 0.1, 95% CI 0.0-0.3, p value <0.001). From the figure, it can be noted that patients with low serum AMH yielded no MII oocytes beyond 12 while 13 (22.0%) of those with normal AMH had more than 15 oocytes harvested. On the whole, more women with normal serum AMH yielded high order of MII oocytes while those with low serum AMH yielded much fewer MII oocytes.

Table 7. Sensitivity, specificity, positive predictive value and negative predictive value of normal versus low serum AMH as a predictor of total oocyte harvest

Serum AMH level	**Total oocyte harvest**		**Total**
	Normal/ High (≥ 5) (Positive)	**Low (<5) (Negative)**	
Normal (Positive)	48	11	59
Low (Negative)	10	16	26
TOTAL	**58**	**27**	**85**

{Sensitivity = 82.8%; Specificity = 59.3%; Positive predictive value (PPV) = 81.4%; Negative predictive value (NPV) = 61.5%}

Sensitivity and positive predictive value of serum AMH as a predictor of total oocyte harvest were both high at 82.8% and 81.4% respectively as depicted in table 7. The specificity and negative predictive value were relatively low at 59.3% and 61.5% respectively.

Table 8. Sensitivity, specificity, positive predictive value and negative predictive value of normal versus low serum AMH as a predictor of Metaphase II oocyte yield

Serum AMH level	**MII oocyte yield**		**Total**
	Normal/ High (≥ 5) (Positive)	**Low (<5) (Negative)**	
Normal (Positive)	43	16	59
Low (Negative)	7	19	26
TOTAL	**50**	**35**	**85**

{Sensitivity = 86.0%; Specificity = 54.3%; Positive predictive value (PPV) = 72.96%; Negative predictive value (NPV) = 73.1%}

As can be seen in table 8, sensitivity, positive predictive value and negative predictive value of serum AMH as a predictor of MII oocyte yield were high (86.0%, 72.96% and 73.1% respectively). However, the specificity was relatively low at 54.3%.

Table 9. MII oocyte yield by age and serum AMH level (N=85)

Age (years)/ Serum AMH	MII oocyte yield		Total	OR(95%CI)	p value
	Low (<5) No(%)	Normal/High (≥5) No(%)			
Normal serum AMH					
<35 yrs	6 (15.4)	33 (84.6)	39	0.2 (0.1-0.8)	0.013
≥35 yrs	9 (45)	11 (55)	20		
Low serum AMH					
<35 yrs	3 (42.9)	4 (57.1)	7	0.1 (0.0-0.9)	0.034
≥35 yrs	16 (84.2)	3 (15.8)	19		

Table 9 shows patients with normal serum AMH levels under the age of 35 years 33 (84.6%) were more likely to have MII oocyte yield of 5 and above. In addition, those with normal AMH levels, those aged ≥35 years were 80% less likely to have normal MII yield compared to those less than 35 years, and was statistically significant with a p-value of 0.013. For patients with low serum AMH who were 35 years and above, 16 (84.2%) had MII oocyte yield of less than 5 compared to 3 (15.8%) with MII oocyte yield of 5 or more. Those patients with normal serum AMH aged below 35 years were 57.1% more likely to have normal/ high MII oocyte yield compared to those with low serum AMH, and was statistically significant with a p value of 0.034.

Table 10. Comparison of reason for cancellation by MII oocyte yield and serum AMH category (N=17)

Reason	Serum AMH category		Total	p value
	Normal No. (%)	Low No. (%)		
Poor response	2 (40)	12 (100)	14	0.003
Hyper response	3 (60)	0	3	
Total	5	12	17	

Table 10 shows the contributors of nil MII oocyte yield by serum AMH category. Poor response as a reason for cancellation was more in patients with low serum AMH 12 (100%) compared to patients with normal serum AMH 2 (40%). Hyper response as a reason for cancellation was seen in 3 (60%) of the patients with normal serum AMH compared to none in patients with low serum AMH. This was statistically significant (p value 0.003). In addition, none of those with normal response in both groups were cancelled.

DISCUSSION

This study exploited the milestones in the sequence that leads to oocyte harvest and MII oocyte yield. These milestones constitute important surrogate indicators of potential oocyte yield. An important milestone of performance in this study includes follicular count, which in this clinic is done on day 5 and day 7 in order to enable gonadotrophin dosage adjustment based on follicular growth, and decisions on continuation with stimulation. On follicular count, throughout the cycle, there is a preponderance of higher count among those who have normal serum AMH compared to those patients with low serum AMH although follicular size was not taken into account, essentially indicating that the difference in response persists throughout the stimulation cycle in subjects with normal and low serum AMH. This was significant for both days 5 and 7 ($p<0.001$). These differentials irrespective of follicular count may be taken as important surrogate indicators of ability to produce fertilizable oocytes. Similar concept has been alluded to, though not in the same context by Brodin T et al who related AMH to live births, qualitative oocyte yield, and embryos[46]. This study entailed a retrospective follow up of a cohort of patients undergoing COS in order to determine the predictive effect of serum AMH levels. To achieve this, the outcomes of OS in women with low and normal serum AMH were compared.

When a cohort of patients undergoes COS, the objective is to get fertilizable oocytes if they are to have a chance of achieving a pregnancy[8]. Therefore, discontinuation either due to poor response or hyper response means the objective is not met thereby affecting the predictive value of serum AMH. This study reveals very high discontinuation rates among the women with low serum AMH with up to nearly 50%, indicating high specificity of low serum AMH levels on prediction of poor response. Out of the total number of patients with normal serum AMH (59), those who had cycle cancellations were 3 (5.08%) and those with poor response were 2 (3.39%). The overall inference of this observation is that serum AMH is a good predictor of ovarian response, the prerequisite of MII oocyte yield. This hyper response however is not excessive, an indication of high margin of safety when serum AMH is normal while at the same time giving advantage of obtaining enough oocytes. Thus, it can be inferred that the likelihood of oocyte harvest is much higher in women with normal serum AMH. Similar concepts have been alluded to by Jayaprakasan K et al who found high predictive value of AMH and AFC to COS[11].

There are currently no standard definitions for normal ovarian response, low response and hyper response, with different authors citing different criteria in their studies[39.47,48,49]. Based on the criteria used in the study of categorization of ovarian response, there was virtually no hyper response among those with low serum AMH, while those with normal serum AMH was 40.7% with total oocyte harvest of 15 or more oocytes. The inference in this instance is that AMH is a good predictor of response to COS though it may not discriminate effectively the risk of hyper response.

In this study, those with normal serum AMH levels were significantly skewed towards having higher follicular count than those with low serum AMH, an important prerequisite to higher MII oocyte yield and therefore ICSI. Hence, this observed skew of increased MII oocyte yield potentiates fertilization, which augurs well with the observation that serum AMH and ovarian response and subsequent CPRs and LBRs [46]. The risk of low response which was less than 5 follicles was high with low serum AMH at 8 (30.8%). The apparent paradox of relative increase in normal response is due to less contribution to hyper response as compared to their counterparts with normal serum AMH. However, a combination of normal and hyper response among the two groups clearly gives the advantage to the women with normal serum AMH who constituted 57 (96.6%) compared to their counter parts with low serum AMH 18 (69.2%) and therefore fulfilling the objective of higher number of oocyte harvest and therefore increased possibility of high level of MII oocytes. Similarly, this is reflected in the results of day 7 in favour of better response among those with normal response. Although the study depicted the impact of age on ovarian stimulation outcomes[24,25], AMH still has predictive value on ovarian response. This was mitigated by standardization of age (under 35 years and 35 years and above) as this difference was maintained after standardization of age in the two categories. Thus, the discriminative effect of serum AMH is seen even after standardizing for age indicating its increased predictive value in MII oocyte yield irrespective of the age. This advantage is also depicted in the data on average indicators of follicular count and total oocyte yield, further supporting the value of serum AMH in predicting COS outcomes.

This study also shows that serum AMH is a good predictor of total oocyte harvest given the high sensitivity, specificity, positive and negative predictive values. Although the specificity was relatively low, it does not dismiss the fact that the subject who opts to undergo COS should be

given a chance albeit with adequate counselling. In addition, the patients should be counselled on the possibility of poor response and cycle cancellation and the possibility of conversion to an oocyte recipient. The high positive predictive value and negative predictive value gives credence to use of serum AMH levels to counsel for good or poor outcomes, particularly given the highly emotive nature of the ART process[46, 52].

Serum AMH is both a predictor of quantity (numbers) and quality (MII oocytes) given the high sensitivity and specificity. The positive and negative predictive values were similarly high. Thus, serum AMH can be used to accurately predict MII oocyte outcomes hence counselling on possible outcomes can be done before the patient undergoes a costly cycle of ovarian stimulation.

In developing countries where ART is expensive and not supported by the health system, the need for cryopreservation of embryos is high[4,5]. Cryopreservation is much cheaper and cost effective as compared to a repeat COS cycle[53]. Hence, serum AMH levels can be used as the platform for counselling on cryopreservation where likelihood for adequate MII oocyte harvest is deemed possible. This study has shown that this possibility can be well predicted where serum AMH is normal and hence enabling advise and counseling on the possibility of cryopreservation in advance.

CONCLUSIONS

1. Normal serum AMH is associated with increased follicular count during stimulation compared to low serum AMH.
2. Normal serum AMH is associated with increased total oocyte harvest during COS compared to low serum AMH.
3. Serum AMH is a good predictor of MII oocyte yield. Hence, serum AMH can be used as a predictor of MII oocyte outcomes in controlled ovarian stimulation cycles.
4. Given the findings of this study, the null hypothesis (H_0) was rejected in favour of the alternate hypothesis (H_1), ***Serum AMH level is a predictor of Metaphase II oocyte yield in controlled ovarian stimulation***.

RECOMMENDATIONS

1. Serum AMH can be used to predict outcomes of OS and hence provide a basis for counselling and advice on outcomes and alternative ART procedures.

2. Need for larger studies using baseline serum AMH levels in our setting to predict with certainty Metaphase II oocytes after COS using modelled receiver operating curves (ROC).

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APPENDICES

APPENDIX I: BUDGET.

ITEM	Kshs
a. Personnel:	
Investigators:	Nil
Data collection per questionnaire	Nil
Data entry	Nil
Data cleaning and analysis	= 20,000.00
SUB –TOTAL	**= 20,000.00**
b. Stationery	
Questionnaire printing	= 10,000.00
Writing materials (papers, pens, pencils)	= 5,000.00
SUB – TOTAL	**= 15,000.00**
c. Transport	
Transport costs @5,000 per month X 3 months	= 15,000.00
SUB – TOTAL	**= 15,000.00**
d. Ethics and review committee	
Ethics and review committee (KNH/UON)	= 2,000.00
SUB-TOTAL	**= 2,000.00**
e. Report writing	
Writing and binding the report	= 10,000.00
Conference presentation and dissemination (FASK AND KOGS)	= 20,000.00
SUB – TOTAL	**= 30,000.00**
TOTAL	**= 82,000.00**
f. Contingency (10%)	**=8,200.00**

GRAND TOTAL = 90,200.00

APPENDIX II: CHRONOGRAM

(PLAN OF ACTIVITY).

ACTIVITY	PROJECT MONTHS											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1. Proposal development	←	—	→									
2. Proposal presentation				←	—	→						
3. Ethical clearance					←	—	→					
4. Pretesting							↔					
5. Data collection							←	—	→			
6. Data analysis										↔		
7. Report writing											←	— →
8. Presentation and dissemination										←	—	→

APPENDIX III: PLAN OF ANALYSIS.

TABLE 1: Selected characteristics of the study population by allotted ovarian response category.

Characteristic	**Study population category**			
Age (years)	**Low response (0.20-0.99 ng/mL)**	**Normal response (1.00-6.80 ng/mL)**	**OR**	**p value**
<20				
20 – 24				
25 – 29				
30 – 35				
> 35				
Type of infertility				
Primary				
Secondary				
Cause of infertility				
Tubal factor				
Ovarian factor				
(Premature ovarian failure)				
Uterine factors				
Male factor				
Stimulation cycle				
First (1)				
Second (2)				
Third (3)				
More than three (>3)				

Table 2: Total Follicular count on day 5 and day 7 by category of serum AMH levels

Day of measurement	**Low response (0.20-0.99 ng/mL)**	**Normal response (1.00-6.80 ng/mL)**	**OR**	**p value**
Day 5				
Low (< 4)				
Normal (4-14)				
Hyper response (>15)				
Day 7				
Low (< 4)				
Normal (4-14)				
Hyper response (>15)				

Table 3: Oocyte yield by levels of serum AMH

Oocyte yield	Low response (0.20-0.99 ng/mL)	Normal response (1.00-6.80 ng/mL)	OR	p value
Total oocyte yield				
< 4				
4-14				
>15				
Metaphase II oocytes yield				
0				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
>12				

Table 4: Proportion of Metaphase II oocyte yield to total oocyte yield

Proportion of M II yield to total oocyte yield
<25%
25 to 50%
51 to 75%
>75%

Table 5: The sensitivity, specificity, positive predictive value and negative predictive value of serum AMH as a predictor of total oocyte yield.

SERUM AMH LEVEL CATEGORY	TRUE STATE (OUTCOME) TOTAL OOCYTE HARVEST		**TOTAL**
	High Harvest (>8) (Positive)	Low (<8) (Negative)	
Normal (Positive)	**A**	**C**	**A+C**
Low (Negative)	**B**	**D**	**B+D**
TOTAL	**A+B**	**C+D**	**A+B+C+D**

Table 6: The sensitivity, specificity, positive predictive value and negative predictive value of serum AMH as a predictor of Metaphase II oocyte yield.

SERUM AMH TEST RESULTS	TRUE STATE		**TOTAL**
	High MII yield (>4)	Low MII yield (<4)	
Normal serum AMH levels (Positive)	**A**	**C**	**A+C**
Low serum AMH levels (Negative)	**B**	**D**	**B+D**
TOTAL	**A+B**	**C+D**	**A+B+C+D**

For each of the tables:

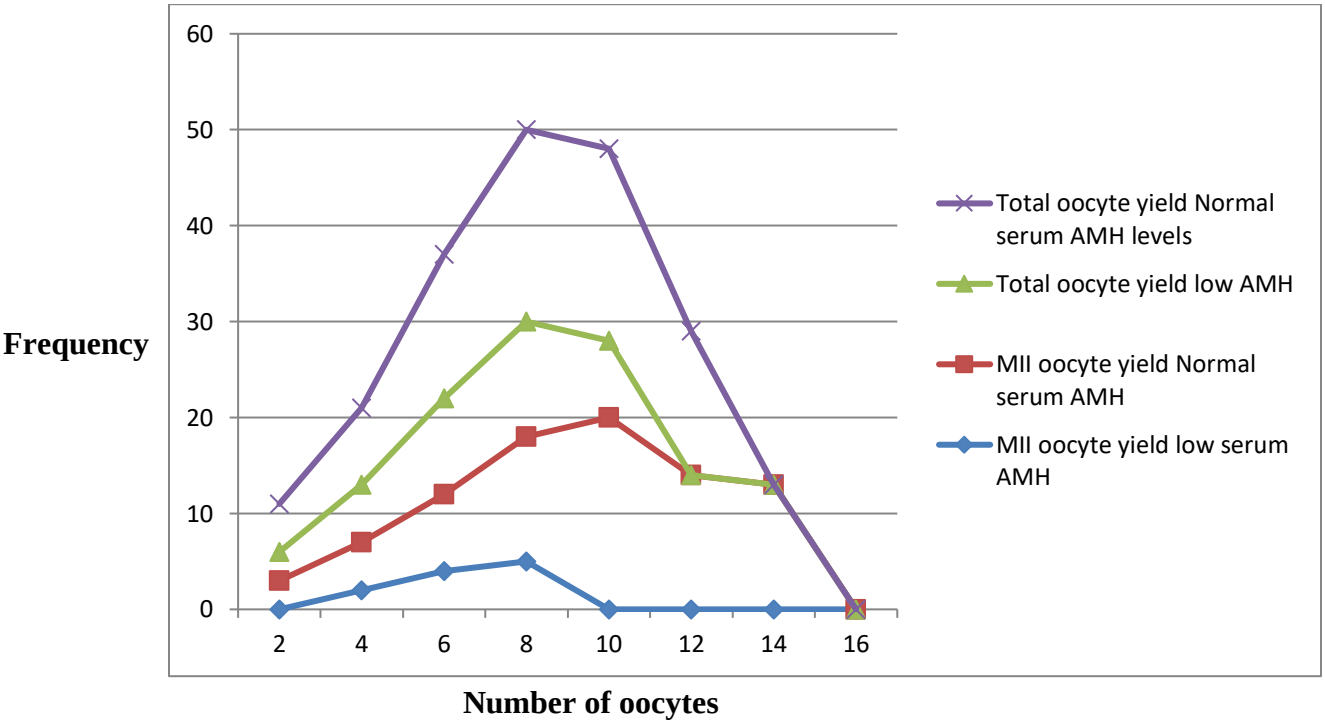
v. **Sensitivity** = $\frac{\mathbf{A}}{\mathbf{A+B}}$ %

vi. **Specificity** = $\frac{\mathbf{D}}{\mathbf{C+D}}$ %

vii. **Positive predictive value (PP)** = $\frac{\mathbf{A}}{\mathbf{A+C}}$ %

viii. **Negative predictive value (NPV)** = $\frac{\mathbf{D}}{\mathbf{B+D}}$ %

Graph 1: The frequency distribution of the patients with normal and low serum AMH by total oocyte and MII oocyte yield.



The above tables are the basic standard tables in the study but other data will be presented to include the comparative means and modes and comparative predictive values, specificity and sensitivity and possibility of cross tabulations by age in particular.

Serum AMH level:
Group A serial number
/____/____/____/

APPENDIX IV: STUDY INSTRUMENT

STUDY INSTRUMENT

Patient number _____

Batch date _____

SECTION A: Essential general characteristics.

Patient's age in completed years: /_____/_____/

AMH levels:

1) In ng/ml: /_____/_____/

Type of infertility:

1) Primary infertility
2) Secondary infertility

Number of ovarian stimulation cycles:

1) None
2) One
3) Two
4) Three
5) More than three

Cause of infertility

1) Tubal factor
2) Ovarian factor
3) Uterine factors
4) Male factor

***SECTION B*: AMH values and categorization of predicted ovarian response.**

AMH levels:

1) In ng/ml: /_____/_____/

Categorization of AMH values:

1) High
2) Satisfactory or optimal
3) Low ☐

***SECTION C:* Follicular count**

Day five (5) follicular count /_____/_____/

Day seven (7) follicular count /_____/_____/

SECTION D: Total oocyte harvest and MII oocyte yield.

Total oocyte yield /_____/_____/

Categorization of oocyte yield

1) Low response (< 4 oocytes)
2) Normal response (5 – 14 oocytes)
3) Hyper response (> 15) ☐

Oocyte yield

1) Total /_____/_____/
2) Metaphase I oocytes /_____/_____/
3) Metaphase II oocyte /_____/_____/
4) Other /_____/_____/

Serum AMH level:
Group B serial number
/____/____/____/

APPENDIX IV: STUDY INSTRUMENT

STUDY INSTRUMENT

Patient number _____

Batch date _____

SECTION A: Essential general characteristics.

Patient's age in completed years: /_____/_____/

AMH levels:

1) In ng/ml: /_____/_____/

Type of infertility:

1) Primary infertility
2) Secondary infertility

Number of ovarian stimulation cycles:

1) None
2) One
3) Two
4) Three
5) More than three

Cause of infertility

1) Tubal factor
2) Ovarian factor
3) Uterine factors
4) Male factor

***SECTION B*: AMH values and categorization of predicted ovarian response.**

AMH levels:

1) In ng/ml: /_____/_____/

Categorization of AMH values:

1) High
2) Satisfactory or optimal
3) Low ☐

***SECTION C:* Follicular count**

Day five (5) follicular count /_____/_____/

Day seven (7) follicular count /_____/_____/

SECTION D: Total oocyte harvest and MII oocyte yield.

Total oocyte yield /_____/_____/

Categorization of oocyte yield

1) Low response (< 4 oocytes)
2) Normal response (5 – 14 oocytes)
3) Hyper response (> 15) ☐

Oocyte yield

1) Total /_____/_____/
2) Metaphase I oocytes /_____/_____/
3) Metaphase II oocyte /_____/_____/
4) Other /_____/_____/

APPENDIX V: OVARIAN STIMULATION PROTOCOL

Ovarian stimulation protocol

The ovarian stimulation protocol used in the Nairobi Fertility Clinic uses the long GnRH agonist protocol. The menstrual cycles are synchronized using combined oral contraceptives a month prior to ovarian stimulation to enable concurrent stimulation in the batch approach. Down regulation is done using long acting goserelin 3.6mgs or long acting leuprolide 3.75mgs administered subcutaneously on the anterior abdominal wall. Down regulation frees the ovary from endogenous gonadotrophins and leaving it at the disposal of exogenous gonadotrophins in OS. The duration of action of these two products is thirty days. Oral contraceptives are stopped two days after giving the long acting GnRH analogue and within a week downregulation bleeding occurs. Approximately 13 to 14 days before the intended last day of transfer, gonadotropins are initialized. Dosage is tailored depending on AMH levels and age in order to achieve individualization and reduce the risk of OHSS. Individualized adjustments are done from day five (5) after assessment of follicular growth in size and numbers by transvaginal ultrasonography. This enables individualized regulation of dosage of gonadotrophins. This is repeated on alternate days and on day nine (9), ten (10) and eleven (11), assessments are done to allot time for trigger if mature eggs are available. The standard trigger is urinary HCG 250 micrograms, done approximately 34 hours after the trigger. Follicular tracking is performed by two specialists and the patients allocated at random. Intra – observer biases are minimal because follicular tracking is easy and high levels of concordance have been demonstrated and, the objective is to achieve enough follicles at the bottom line – measuring 17 to 20 millimetres.

IVF Laboratory procedures

All of the laboratory procedures are done by one very qualified embryologist. The following steps are followed:

i. After aspiration, oocyte grading and stripping of granulosa cells is done under inverted microscope and includes identification of Metaphase I oocytes and MII oocytes.

ii. All of them are kept in the oocyte media in the incubator at 37.0 degrees with air saturated with carbon dioxide in order to maintain pH at physiological levels for up to 2 hours. During this period, some of the Metaphase I (MI) oocytes covert to MII oocytes in the media.

iii. ICSI is universally used for fertilization. It is done on MII oocytes.

iv. The MII oocytes are incubated at 37.0 degrees centigrade for 24 hours after which assessment of fertilization and cleavage is done using morphological classification. Embryo transfers are done on either day two (2) or three (3) depending on level of embryo development.

v. Luteal phase support is given using vaginal and oral progesterone until day fourteen (14) when serum beta HCG is done.

APPENDIX VI: LETTER TO THE DIRECTORS.

Dr. Mary Kiria Koigi
P.O. BOX 19784-00202,
NAIROBI.
27TH February 2019

To:
The Directors of Nairobi Fertility Clinic,
Nairobi.

ATTN: PROF. KOIGI KAMAU AND DR. WANYOIKE GICHUHI

Dear Sir,

RE: PERMISSION TO USE CLINIC DATA FOR MY MASTERS THESIS.

I wish to request for permission to use raw data from the Nairobi Fertility Clinic patient's records for my masters dissertation entitled '**SERUM ANTI-MULLERIAN HORMONE AS A PREDICTOR OF METAPHASE II OOCYTE YIELD DURING CONTROLLED OVARIAN STIMULATION IN A PRIVATE FERTILITY CLINIC IN NAIROBI'**. Your assistance on this matter will be highly appreciated.

Yours Faithfully,

Dr. Mary Kiria Koigi
Resident MMedObs/Gyn,
Year 2.