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UMST UNIVERSITY
جامعة العلوم الطبية والتكنولوجيا
UMST University of Medical Sciences & Technology
P. O. Box 12810,Khartoum, Sudan
T. +249 183 228614
F. +249 183 224799
E. administration.office@umst-edu.sd
W. www.umst-edu.sd

University of Medical Sciences & Technology
Faculty of Medicine
Department of Research Methodology & Biostatistics

Prevalence of Female Infertility based on Anti-Müllerian Hormone Level in Royal Care Hospital, Khartoum 2020-2021

Submitted in partial fulfillment for the MBBS degree

By

Ahmed Hamdi Abdulgafar Khalfalla
MD-2017-050
University of Medical Sciences and Technology
Academic year 2021-2022
Batch 24 – 2017

Supervised by

Dr. Mohamed Hafiz Salim

Consultant of Obstetrics and Gynaecology

Royal Care Fertility Clinic

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Faculty of Medicine

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ABSTRACT

Background: Anti-Müllerian hormone (AMH) is a dimeric glycoprotein and a member of the transforming growth factor β (TGF-β) family of growth and differentiation factors. AMH is a product of the granulosa cells from the pre-antral and small antral follicles. It acts as a gate keeper of small growing follicles, limiting follicular growth initiation, and subsequently oestradiol production from small antral follicles prior to selection. AMH is used as an endocrine marker for ovarian aging in normogonadotropic and anovulatory infertile women. Due to the prevalence of infertilitywhich is a neglected issue, particularly in poorer nations, substantial study is required.

Objective: To determine the prevalence of female infertility based on Anti -Müllerian hormone level to correlate between anti-müllerian hormone, follicle stimulating hormone and Estradiol levels. To assess the correlation between age of female and levels of anti-müllerian hormone.
Methods: This study was done as a facility-basedRetrospective, descriptive study design, at Royal Care international hospital, with a number of patients who underwent ICSI for the fertility centre.

Results: For the type of infertility 67 are having primary infertility (58.3%), 48 have a secondary one (41.7%), for the FSH level 60 have a high level (52.2%), 54 have a normal level (47%), 1 has a low level (0.9%), for the AMH level 114 have a low one (99.1%), 1 has a normal level (0.9%).For the number of the previous ICSI attempts 71 had none (61.7%), 20 had one (17.4%), 13 had 2 (11.3%), 5 had 5 (4.3%), 4 had 3 (3.5%), 2 had four times (1.7%), for the outcome of previous ICSI 107 had 0 (negative) (93%), 8 had one (0.7%), for the protocol for ovarian stimulation 102 got HMG (88.7%), 9 got FSH (7.8%), 4 had natural cycle (3.5%), for the number of follicles produced 96 had 1-3 (83.5%) follicles, 18 had 4-6 (15.7%) follicles, 1 had from 7-10 (0.9%) follicles.

Conclusion: In conclusion 115 couples presented to the Fertility Centre of Royal care international hospital in the study period. Respectively 58% had primary infertility and 42% had secondary infertility. In summary the study found no correlation between age and AMH, and no correlation between AMH, FSH and estradiol levels. On the other hand, a strong correlation between AMH and number of oocytes produced. Moreover, a strong correlation between AMH and success rates of ICSI was established. As so concurring with international studies in addition of being a strong indicator of premature ovarian failure.

Keywords:- *AMH, Infertility, ICSI.*

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DEDICATION

I dedicate this to my parents who without their support and care I wouldn't have achieved anything and for being my strongest source of motivation. To my younger brothers who will always be my source of peace and the reason I push for perfection to set a great role model for them. To all myfriends and extended family who are always there for me when I need them the most. Finally, to all my teachers who enriched me with knowledge and discipline to shape my future.

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Firstly, Alhamdulillah SWT for the patience, knowledge, and strength He bestowed upon me to finish this work. A sincere thanks to my supervisor Dr. Mohammed Hafiz and Dr. Hiba for their continuous support and precious time making this a smooth learning journey. I would like to also thank everybody who assisted and helped me out in every step of the way.

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LIST OF ABBREVIATIONS

ICSI	Intracytoplasmic Sperm Injection (ICSI)
UMST	University of Medical Science and Technology
AMH	Anti-Müllerian hormone
ART	assisted reproductive technologies
ELISA	enzyme-linked immunosorbent assays
IVF	Intra vitro fertilization
FSH	Follicular Stimulating hormone
COS	Controlled Ovarian Stimulation
AFC	Antral Follicle Count

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CHAPTER ONE

INTRODUCTION

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein and a member of the transforming growth factor β (TGF-β) family of growth and differentiation factors. AMH is a product of the granulosa cells from the pre-antral and small antral follicles therefore its measurable in the plasma. It acts as a gate keeper of small growing follicles, limiting follicular growth initiation, and subsequently estradiol production from small antral follicles prior to selection. When the growing follicles reach a suitable size and differentiation state, they are selected for dominance by action of FSH secreted by the pituitary.[1][2]

Levels of AMH in women are used to illustrate the ovarian follicular pool and they are beneficial in reflecting the ovarian reserve. Clinically, AMH measurements could potentially predict the quantitative and qualitative aspects of assisted reproductive technologies (ART).[2]

The ovarian reserve is made up by the size of the ovarian follicle pool and the quality of the oocytes there in, which drops as age advances resulting in a decrease of woman's reproductive function.[1] A patient's Ovarian reserve determines prognostic chances of fertility treatments.

Infertility is the inability to conceive (regardless of cause) after 1 year of unprotected intercourse. Its overall prevalence has been stable during the past 50 years; however, a shift in etiology and patient age has occurred. As woman's age increases, the incidence of infertility also increases.[3]

The benefits of AMH clinically are numerous. It can act as peripheral signal of the ovarian pool as mentioned previously. For an instance in women undergoing fertility treatment, ovarian aging is considered by diminished ovarian responsiveness and a poor pregnancy outcome. On the contrary, the right identification of poor responders by assessment of the ovarian reserve may also benefit other patients who are omitted because of advanced age.[1]

Over the last decades the possibility of reflecting the ovarian reserve via the ovarian reserve markers such as AMH was researched thoroughly as low ovarian reserve may be an important cause of infertility. These further assists clinicians to predict the prognosis of COS in IVF cycles: if a patient has a low ovarian reserve, she will probably receive a poor ovarian response after COS characterized by a low number of follicles and low serum oestradiol levels after exogenous gonadotropin stimulation, resulting in a poor oocytes retrieval and often in a poor reproductive outcome. However, this prognosis may also be prompted by a plethora of other variables such as patient's age and the outcome of previous ICSI trials. [4]

Some of these ovarian reserve markers are serum FSH, serum AMH, estradiol and the AFC. Serum FSH has been used extensively in reproductive medicine and is measured in the early follicular phase (days 3-5 of the menstrual cycle) along with estradiol. However, it is only an indirect marker of ovarian reserve, and its blood concentrations increase only when ovarian reserve is severely depleted.[5]AFC is performed by ultrasound and counts all identifiable antral follicles of 2–10 mm present in both ovaries.[6]AMH has the benefit of having significantly minimal intra- and inter-cycle variability when compared to AFC.[7]

Due to this, the European Society of Human Reproduction and Embryology (ESHRE) consensus has stated that a response can be described as poor ovarian response when at least two of the following conditions are meet: (i) advanced femaleage, (ii) a prior poor ovarian response, (iii) an abnormal ovarian reserve testor, in the absence of the aforementioned requirements, two prior PORfollowing maximal stimulation.[8]Nevertheless, certain certain characteristics concerning some demographic, biological and clinical features among patinets included in the previous definition of poor ovarian response were neglected this was further improved by the Poseidon classification which stratified subgroups based on their characteristics.[9]

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Two commercial enzyme-linked immunosorbent assays (ELISAs) have been available since 2004 one from immunotech (France) and the other from Diagnostic systems laboratory (USA). AN international reference standard is yet to be established, this would make comparing assays more reliable and therefore applications more accurate.[10]

A. Problem Statement:

Women's ability to conceive a child is a very important matter to study. In today's day and age women choose to delay their pregnancies and child birth in concordance with their needs. Therefore, a more accurate technique to calculate the ovarian reserve is needed by reproductive medicine practitioners. Anti-müllerian hormone makes a perfect candidate for this role but not alone. Several factors such as age, follicle stimulating hormone levels should complement anti-müllerian hormone to fulfil its prognostic value.

B. Justification:

Fertility professionals use anti-müllerian hormone as a prognostic in the management of their patients and a diagnostic in their protocols. To acquire knowledge whether anti-müllerian hormone is of a prognostic value further research on its effect on female infertility is required [1]. This research aims to link the knowledge known about premature ovarian failure and ovarian sufficiency to the levels of anti-müllerian hormone in addition to amend the category of poor responders on bases of the anti-müllerian hormone.

C. Research Question:

Does Anti-Müllerian hormone have a significant incidence on the diagnosis of female infertility?

D. Objectives:

- *General Objectives:*
  To determine the prevalence of female infertility based on Anti -Müllerian hormone level

- *Specific Objectives:*

- To correlate between anti-müllerian hormone , follicle stimulating hormone and Estradiol levels.
- To assess the correlation between age of female and levels of anti-müllerian hormone.
- To assess the relationship between the levels of anti-müllerian hormone and number of oocytes produced.
- To assess the link between anti-müllerian hormone levels and the success rate of ICSI (intracytoplasmic sperm injection).
- To determine if anti-müllerian hormone respectively is an indicator of premature ovarian failure.
- To determine the best protocol for management in poor responders.

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CHAPTER TWO

LITERATURE REVIEW

In a study conducted in Rotterdam, The Netherlands in the year 2006 by Jenny A visser, Frank H de Jong, Joop S E Laven and Axel P N Themmen titled "Anti-Müllerian hormone: a new marker for ovarian function". It was concluded that the examining of the ovarian reserve is predominantly important in the IVF clinic, AMH was concluded to be a beneficial prognostic of poor response as numerous cases of subfertility is due to adjournment of childbearing. Nevertheless, this study determined that to obtain knowledge on whether serum AMH levels has prognostic value much more prospective studies in normal population are required to provide solid evidence of this concept.[1]

In another article published in the year 2018 by Shunpig Wang et al, studying the roles of AMH and FSH in predicting live birth in patients with discordant AMH and FSH. They implemented a retrospective study using data from eIVF consisting of 13,964 cycles with AMH, FSH, age, BMI, and birth outcomes were evaluated. Patients were broken down into four groups: Good prognosis group (AMH ≥1 ng/ml; FSH < 10 mIU/ml), Poor prognosis group (AMH < 1 ng/ml; FSH ≥10 mIU/ml), Reassuring AMH group (AMH ≥1 ng/ml; FSH ≥10 mIU/ml), and Reassuring FSH group (AMH < 1 ng/ml; FSH < 10 mIU/ml). The interaction between AMH, FSH, and their impact on live birth rate among these four groups was assessed using Generalized Additive Mixed Modelling (GAMM). The good prognosis group had the highest live birth rate while the poor prognosis group had the lowest live birth rate (29.3% vs 13.1%, $p < 0.005$). In the discordant groups, the live birth rate of the reassuring AMH group was significantly higher than the reassuring FSH group (22.8% vs 15.6%, $p < 0.005$). The result of the study showed a nonlinear relationship of AMH and FSH with live birth rate among all the 4 groups. However, all after the results finding that AMH is superior to FSH as clinical predictor of cycle success especially in situations of discordant results, they concluded that neither AMH nor FSH alone can be used as a prognostic in infertility patients. An entire analytical model should integrate these markers along with the patient's demographics and treatment response to provide a more precise prognostic direction.[11]

In a study conducted in the Department of obstetrics and gynaecology by Grynnerup AG et al, at Roskide Hospital, University of Copenhagen, in the year 2012, Studying the role of AMH in female's fertility and infertility. It was concluded that AMH in addition to other endocrine markers are frail indicators to withhold IVF treatment and it is not practical financially to suggest that a woman should undergo IVF treatment based exclusively on low AMH levels. However, AMH levels along with other factors such as age could be used as a pre-treatment counselling reference. Moreover, increasing oocyte yield is based on AMH levels in poor responders is not clear as not enough research have been done supporting the strategy and the published results on the topic have been ambiguous.[12]

In a retrospective analysis study by Benjamin Leader et al in 2012. Which took place in 30 United states fertility centres, to determine the frequency of clinical discordance between anti-müllerian hormone and follicular stimulating hormone using clinical cut points defined by the controlled ovarian stimulation in the same serum samples taken on days 2 to 4 of the menstrual cycle (estradiol was used to confirm the cycles). This study aimed to ultimately answer the question of "how often one hormone is reassuring when the other is concerning". The results have shown that this happened quiet regularly with approximately 1 in 5 women overall when using clinical cut points defined by the risk of poor ovarian response. Moreover, concerning AMH values were observed in 1 in 5 women with reassuring FSH values in a highly age-dependant fashion, ranging from 1 in 11 women under 35 years of age to 1 in 3 women above 40 years of age. The widespread occurrence of AMH and FSH discordance suggests that relying solely on early follicular FSH and estradiol levels to test for poor ovarian reserve would lead to many women getting false reassurance. This false reassurance arises in part because the reduction in AMH occurs before the rise in FSH. Furthermore, previous studies using the same laboratory developed AMH assay have indicated that

AMH has a higher sensitivity for diagnosing poor ovarian response than FSH. It concluded that this discordancy has clinical significance in fertility care, and that each hormone is used differently in relation to other important clinical conditions, it seems reasonable to measure both FSH and AMH in all age groups for a comprehensive female fertility evaluation. However, demographic and treatment outcomes in future studies should be incorporated to further develop and utilize the implantation of both hormones in female fertility care.[13]

In a cohort study in 2021, in the reproductive medical center in Peking university hospital of Beijing, China by Tie-Cheng Sun et al. Trying to investigate if increased anti-müllerian hormone concentration is a useful tool to predict the outcome of assisted reproductive treatment. The study involved 520 patients who underwent IVF/ICSI procedures in the university hospital. Furthermore, they measured the serum AMH level on day 3 of the cycle and based on that divided them into three groups namely low, average, and high. This study found that elevated AMH levels had no correlation with the number of clinical pregnancy rates. This because AMH can reflect the number of follicles but cannot rule out quality of these follicles.As a result, even when more good quality embryos are utilized, people with greater AMH levels may still be unable to conceive. The amount of good quality embryos is used in most IVF and ICSI regimens to boost success rates. More good quality embryos, on the other hand, may not always imply better assisted reproductive technique results. As a result of these findings, one of the concerns that emerges is that AMH levels are not an independent predictor of the number of clinical pregnancy rates for IVF/ICSI cycles.[14]

In a retrospective cohort study by Xiao-Ling GU et al conducted by in 2022 involving women who have undergone 335 cycles in the period between January 2019 to December 2020 at a Nantong affiliated hospital in China. This study was trying to explore the effect of AMH on embryonic development by studying the relationship between serum AMH concentration and the high-quality embryo number during the first cycle of IVF/ICSI in 30–44-year-old infertile women. Their methods were to collect the demographics, clinical medications, and cycle outcomes of these patients and find a correlation between AMH and the number of high-quality embryos by analysing the results. The results of this study again confirmed that AMH has an extremely high specifity, sensitivity and is negatively correlated with age. In addition to this AMH stability between cycles as well as the convenience of AMH test makes it an ideal clinical biomarker for the ovarian response to controlled ovarian stimulation. This study also improved on the idea that AMH can predict not only the number but also the quality of embryos. They reached such a conclusion by performing a generalized additive model analysis and a two-piecewise regression model, the results demonstrated a nonlinear relationship between AMH and the number of high-quality embryos in addition to a not significant relationship. However, after adjusting for confounding factors such as female age and controlled over hyperstimulation in a stratified analysis interaction test, the positive correlation between AMH and the number of high-quality embryos was stable. Finally, this study concluded that a younger female, lower FSH and higher antral follicle count are related with higher AMH, this is consistent with previous papers that concluded that AMH was negatively correlated with age and FSH, and positively correlated with antral follicle count. Therefore, the effect of age-related ovarian reserve biomarker AMH on reproductive prognosis can provide reference for ART clinical treatment.[15]

In a facility based retrospective study by Tie-Cheng Sun et al in 2022 based in Peking University People's Hospital, 521 infertile women were recruited retrospectively aged between 22-43 years from the period between September 2015 to February 2017. They collected data including the maternal age, reproductive hormonal profiles including the AMH, FSH and others. They excluded all women under medication such as clomiphene and letrozole in the last 12 weeks and women with endocrine or autoimmune diseases. What makes this study more accurate is the standardization of ovarian stimulation protocol as all women received a standard luteal downregulation regimen, flare-up short regimen and gonadotropin antagonist protocols. This study concluded that a decreasing trend in antral follicle count, AMH, antral follicle count to age ratio and AMH to age ratio with an increase in age of female has a positive correlation with the number of oocytes retrieval and good quality embryos which suggest that these values have a high predictive value in terms of ovarian function especially in fertile women of advanced age.[16]

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In a retrospective cohort study conducted in July 2022 by P. Romanski et al at the Perelman and Claudia Cohen centre for reproductive medicine in New York assessing if low AMH level is negatively associated with pregnancy outcomes intrauterine insemination cycles. This study argued that before IVF either ovulation induction or intrauterine insemination cycles in a patient with sufficient ovarian reserve is normally trialled. however, in those with diminished reserves advancing straight to IVF due to concerns regarding intrauterine insemination cycles which may yield unacceptable pregnancies and live birth rates is the norm in clinician's protocols which is quite not accurate. In this study patients were grouped according to their AMH levels respectively AMH $\geq$ 1.0 and AMH <1.0, their results concluded that no significant difference was found in clinical pregnancy, ongoing pregnancies, and pregnancy rates in both groups even after adjusting for other limitations. Therefore, this assists clinicians to trial intrauterine insemination cycles or ovulation induction regardless of the AMH level as its not associated with chances of success or failure.[17]

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CHAPTER THREE

MATERIAL AND METHODS

A. STUDY DESIGN

It was a facility-basedRetrospective, descriptive study design.

B. STUDY AREA AND POPULATION

- Study Area

Royal care fertility centre Royal Care International Hospital, Burri, Khartoum Locality, Khartoum State, Sudan.

- Study population

All the female patients attending the Royal care fertility centre in the period between 2020 till 2021.

C. SAMPLE SIZE AND SAMPLING TECHNIQUE

Total coverage of female patients undergoing infertility treatments and protocols.

- *1Inclusion criteria*

All the female patients attending the Royal care fertility centre in the period between 2020 till 2021.

- *Exclusion criteria*

Patients who undergo mature oocyte cryopreservation and Patients who can not undergo the ICSI procedure.

- *Variables:*

Age, AMH, FSH, ICSI attempts and their outcome, Number of follicles produced, Estradiol level on day of HCG, Number of oocytes retrieved, Number of embryos introduced.

D. DATA COLLECTION, MANAGEMENT AND STATISTICAL ANALYSIS

- *Data Collection Tools*

Data was collected via the use of a checklist from the patient's files and Clinical data were obtained using a data sheet from a review of electronic medical records / patients file and charts.

- *Data management and statistical analysis*

The data was analyzed through SPSS software version 26, The results were generated in the form of charts, tables and graphs.

E. Ethical Consideration:

This study was sought from the research technical and ethical committee at the Faculty of Medicine, informed consent from the management of the targeted hospital (Royal care hospital), patient's privacy and confidentiality were maintained by ensuring that no patient names or contact were included in the study. Also the ethical consent was taken from the Faculty Administration

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CHAPTER FOUR

RESULTS

For the Date distribution: 13 are in Jan 2020 (11.3%), 10 are in Sep 2020 (8.7%), as same as Mar 2021, 9 are in Oct 2021 (7.8%), 8 are in Mar and Nov 2021 (7%), 7 are in Dec 2020, Apr 2021, June 2021 and Sep 2021 (6.1%), 6 are in Jan 2021, and Aug 2021 (5.2%), 5 are in Oct 2020 (4.3%), 4 in Feb 2020 (3.5%), 3 in June 2020 and July 2020 (2.6%), 2 in Nov 2020 (1.7%).

Date Distribution		Frequency	Percent
	JAN 2020	13	11.3
	SEP 2020	10	8.7
	MAR 2021	10	8.7
	OCT 2021	9	7.8
	MAR 2020	8	7.0
	NOV 2021	8	7.0
	DEC 2020	7	6.1
	APR 2021	7	6.1
	JUN 2021	7	6.1
	SEP 2021	7	6.1
	JAN 2021	6	5.2
	AUG 2021	6	5.2
	OCT 2020	5	4.3
	FEB 2020	4	3.5
	JUN 2020	3	2.6
	JUL 2020	3	2.6
	NOV 2020	2	1.7
	Total	115	100.0

Table 4.1: The Date Distribution

For the Age distribution: 62 are aged between 30-40 years old (53.9%), 47 are older than 40 (40.9%), 6 in between 20-29 years old (5.2%).

Age Distribution		Frequency	Percent
Valid	**From 30-40**	62	53.9
	Older than 40	47	40.9
	From 20-29	6	5.2
	Total	115	100.0

Table 4.2: The Age Distribution

For the type of the infertility 67 are having primary infertility (58.3%), 48 have a secondaryinfertility (41.7%), for the FSH level 60 have a high level (52.2%), 54 have a normal level (47%), 1 has a low level (0.9%), for the AMH level 114 have a low level (99.1%), 1 has a normal level (0.9%).

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		Count	Table N %
Type of infertility:	**Primary**	67	58.3%
	Secondary	48	41.7%
FSH Level	**High**	60	52.2%
	Normal	54	47.0%
	Low	1	0.9%
AMH Level	**Low**	114	99.1%
	Normal	1	0.9%

Table 4.3: The Data regarding the infertility, FSH and AMH levels:

For the number of the previous ICSI attempts 71 had none (61.7%), 20 had one (17.4%), 13 had 2 (11.3%), 5 had 5 (4.3%), 4 had 3 (3.5%), 2 had four times (1.7%), for the outcome of previous ICSI 107 had 0 (negative) (93%), 8 had one (0.7%), for the protocol for ovarian stimulation 102 were given HMG (88.7%), 9 were given FSH (7.8%), 4 had a natural cycle (3.5%), for the number of follicles produced 96 had 1-3 follicles (83.5%), 18 had 4-6 follicles (15.7%), 1 had from 7-10 follicles (0.9%).

		Count	Table N %
No. of previous ICSI attempts:	**0**	71	61.7%
	1	20	17.4%
	2	13	11.3%
	5	5	4.3%
	3	4	3.5%
	4	2	1.7%
Outcome of previous ICSI:	**0 -ve**	107	93.0%
	1	8	7.0%
Protocol for ovarian stimulation:	**HMG**	102	88.7%
	FSH	9	7.8%
	Natural cycle	4	3.5%
No. of follicles produced:	**1-3**	96	83.5%
	4-6	18	15.7%
	7-10	1	0.9%

Table 4.4: The Data regarding the ICSI:

For the Estradiol Levels on day of HCG 78 had 200-599 (67.8%), 19 had less than 200 (16.5%), 11 had from 600-1000 (9.6%), 7 had more than 1000 (6.1%), for the number of oocytes retrieved 80 retrieved from 1-3 oocytes (69.8%), 33 retrieved no oocytes (28.7%), 1 retrieved from 7-10 oocytes (0.9%) and also 1 retrieved from 4-6 oocytes. For the number of embryos introduced 53 had no embryos introduced (46.1%), 47 had one embryo introduced (40.9%), 12 had two embryos introduced (10.4%), 2 had three embryos introduced (1.7%), 1 had four embryos introduced (0.9%), for the outcome of the ICSI 49 did not get pregnant (42.6%), 44 had no ICSI (38.3%), 22 got pregnant (19.1%).

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		Count	Table N %
Estradiol level on day of HCG:	**200-599**	78	67.8%
	<200	19	16.5%
	600-1000	11	9.6%
	More than 1000	7	6.1%
No. of oocytes retrieved:	**1-3**	80	69.6%
	0	33	28.7%
	7-10	1	0.9%
	4-6	1	0.9%
No. of embryos introduced:	**0**	53	46.1%
	1	47	40.9%
	2	12	10.4%
	3	2	1.7%
	4	1	0.9%
Outcome of ICSI:	**Not pregnant**	49	42.6%
	NO ICSI	44	38.3%
	Pregnant	22	19.1%

Table 4.5: The data regarding the Oocytes retrieved:

Type of infertility: * FSH Level Crosstabulation						
			FSH Level			**Total**
			High	**Low**	**Normal**	
Type of infertility:	**Primary**	**Count**	40	1	26	67
		% Of Total	34.8%	0.9%	22.6%	58.3%
	Secondary	**Count**	20	0	28	48
		% Of Total	17.4%	0.0%	24.3%	41.7%

Table 4.6: Comparison between the type of infertility and the FSH levels
P Value of 0.094

Type of infertility: * AMH Level Crosstabulation					
			AMH Level		**Total**
			Low	**Normal**	
Type of infertility:	**Primary**	**Count**	66	1	67
		% Of Total	57.4%	0.9%	58.3%
	Secondary	**Count**	48	0	48
		% Of Total	41.7%	0.0%	41.7%

Table 4.7: Comparison between the type of infertility and the AMH levels:

P value of 0.395

Type of infertility: * Oestradiol level on day of HCG: Crosstabulation							
			Estradiol level on day of HCG:				**Total**
			<200	**200-599**	**600-1000**	**More than 1000**	
Type of infertility:	**Primary**	**Count**	14	40	7	6	67
		% of Total	12.2%	34.8%	6.1%	5.2%	58.3%
	Secondary	**Count**	5	38	4	1	48
		% of Total	4.3%	33.0%	3.5%	0.9%	41.7%

Table 4.8: Comparison between the type of infertility and the Estradiol levels:
P Value of 0.126

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Age Distribution * FSH Level Crosstabulation						
			FSH Level			Total
			High	Low	Normal	
Age Distribution	From 20-29	Count	3	0	3	6
		% Of Total	2.6%	0.0%	2.6%	5.2%
	From 30-40	Count	33	1	28	62
		% Of Total	28.7%	0.9%	24.3%	53.9%
	Older than 40	Count	24	0	23	47
		% Of Total	20.9%	0.0%	20.0%	40.9%

Table 4.9: Comparison between the age and the FSH levels:
P Value of 0.913

Age Distribution * AMH Level Crosstabulation					
			AMH Level		Total
			Low	Normal	
Age Distribution	From 20-29	Count	6	0	6
		% Of Total	5.2%	0.0%	5.2%
	From 30-40	Count	61	1	62
		% Of Total	53.0%	0.9%	53.9%
	Older than 40	Count	47	0	47
		% Of Total	40.9%	0.0%	40.9%

Table 4.10: Comparison between the age and the AMH levels:
P Value of 0.650

AMH Level * No. of follicles produced: Crosstabulation						
			No. of follicles produced:			Total
			1-3	4-6	7-10	
AMH Level	Low	Count	95	18	1	114
		% Of Total	82.6%	15.7%	0.9%	99.1%
	Normal	Count	1	0	0	1
		% Of Total	0.9%	0.0%	0.0%	0.9%

Table 4.11: Comparison between the AMH and No. of follicles produced:
P Value of 0.905

AMH Level * No. of oocytes retrieved: Crosstabulation							
			No. of oocytes retrieved:				Total
			0	1-3	4-6	7-10	
AMH Level	Low	Count	33	79	1	1	114
		% Of Total	28.7%	68.7%	0.9%	0.9%	99.1%
	Normal	Count	0	1	0	0	1
		% Of Total	0.0%	0.9%	0.0%	0.0%	0.9%

Table 4.12: Comparison between the AMH and No. of Oocytes retrieved:

P Value of 0.093

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AMH Level * No. of embryos introduced: Crosstabulation								
			No. of embryos introduced:					Total
			Zero	One	Two	Three	Four	
AMH Level	Low	Count	53	46	12	2	1	114
		% Of Total	46.1%	40.0%	10.4%	1.7%	0.9%	99.1%
	Normal	Count	0	1	0	0	0	1
		% Of Total	0.0%	0.9%	0.0%	0.0%	0.0%	0.9%

Table 4.13: Comparison between the AMH and No. of embryos introduced:
P Value of 0.00

AMH Level * Outcome of ICSI: Crosstabulation						
			Outcome of ICSI:			Total
			NO ICSI	Not pregnant	Pregnant	
AMH Level	Low	Count	44	48	22	114
		% Of Total	38.3%	41.7%	19.1%	99.1%
	Normal	Count	0	1	0	1
		% Of Total	0.0%	0.9%	0.0%	0.9%

Table 4.14: Comparison between the AMH and outcome of the ICSI:
P Value of 0.01

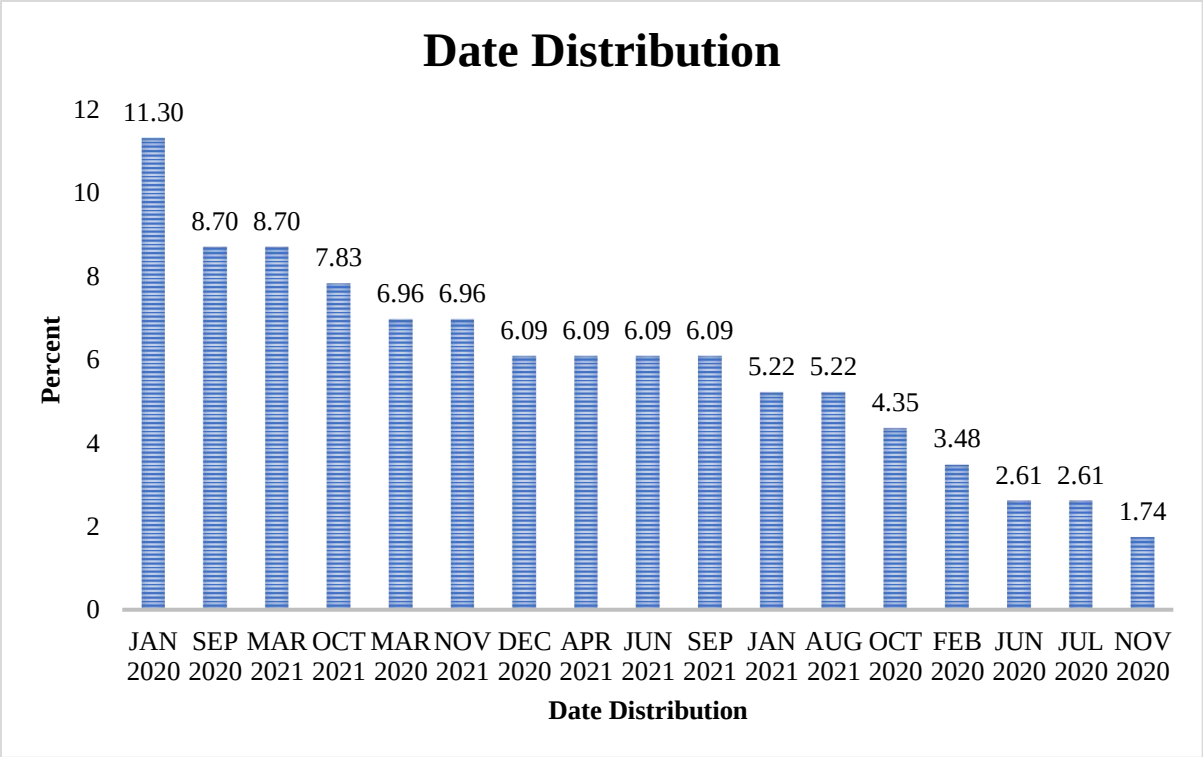

Fig. 4.1: The Dates Distribution.

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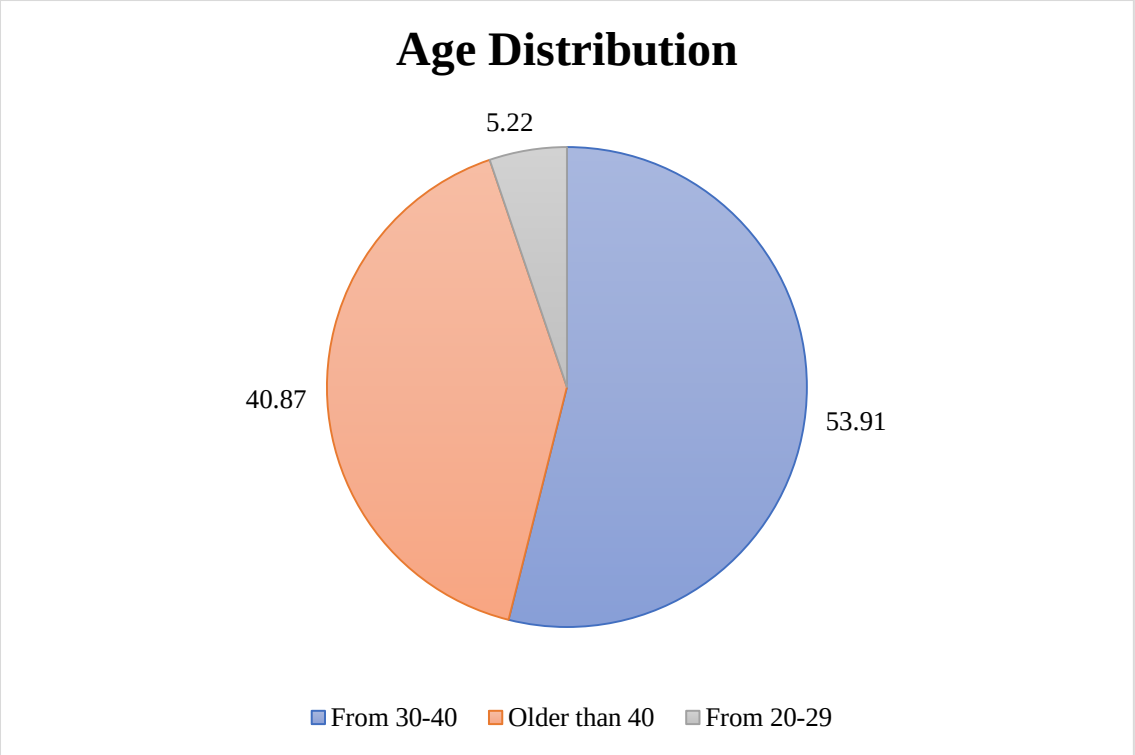

Fig. 4.2: The age distribution.

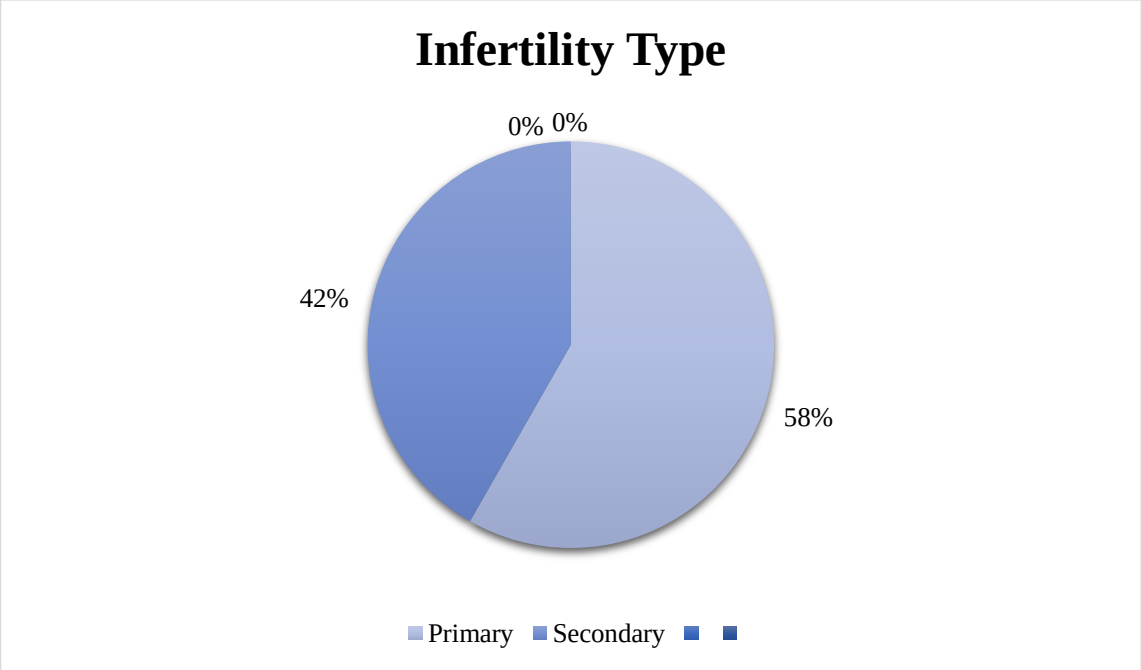

Fig. 4.3: Infertility type.

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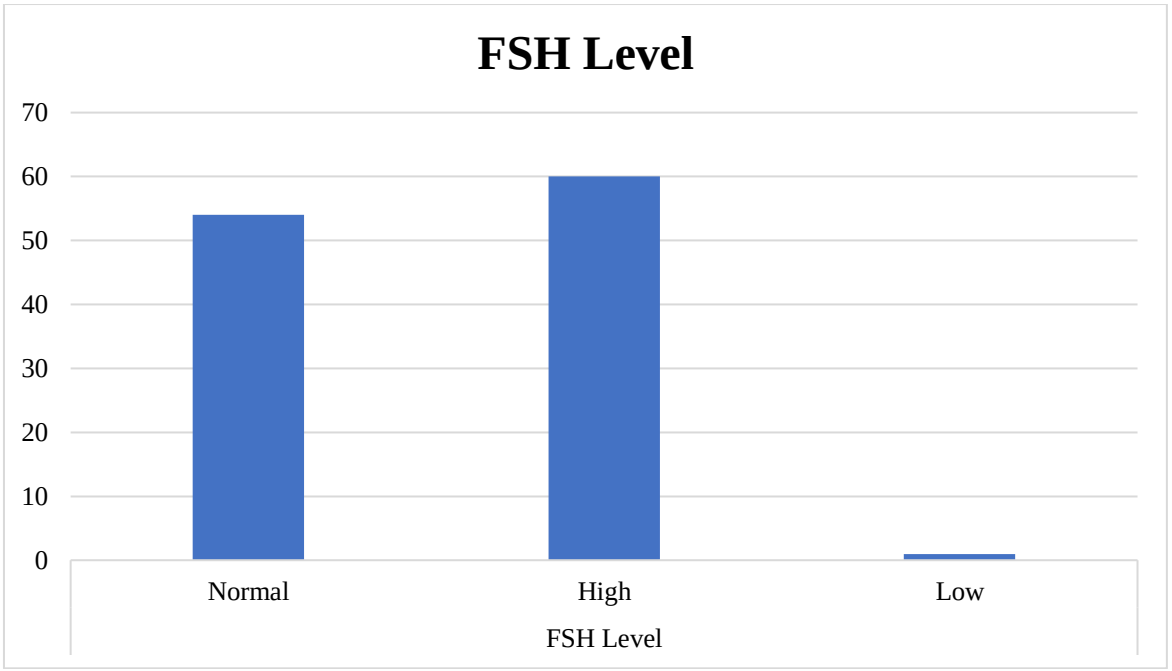

Fig.e 4.4: TheFSH Level

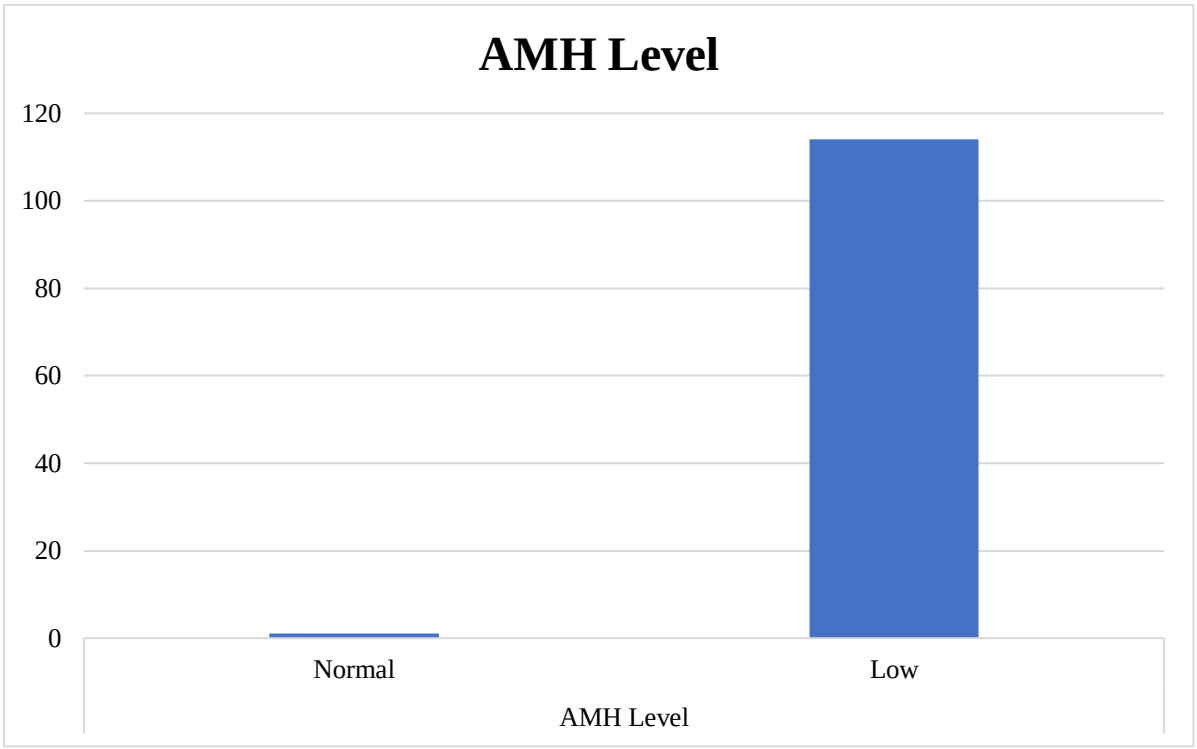

Fig. 4.5: The AMH level.

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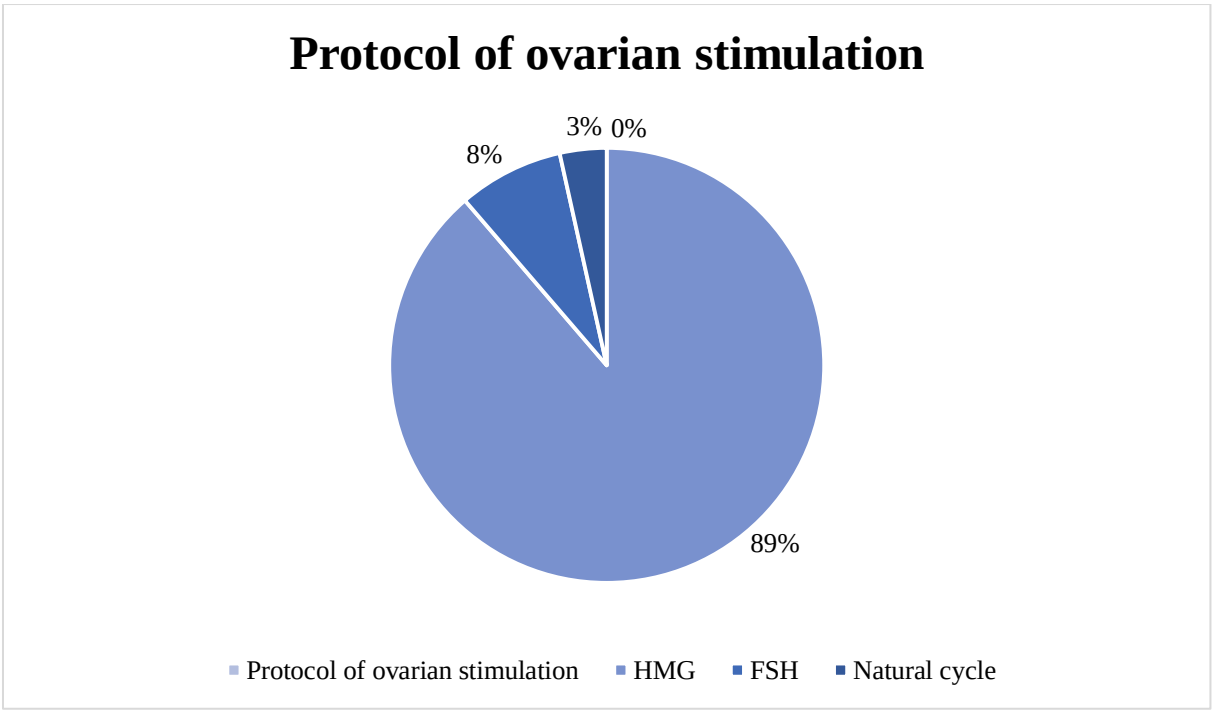

Fig. 4.6: Protocol of ovarian stimulation.

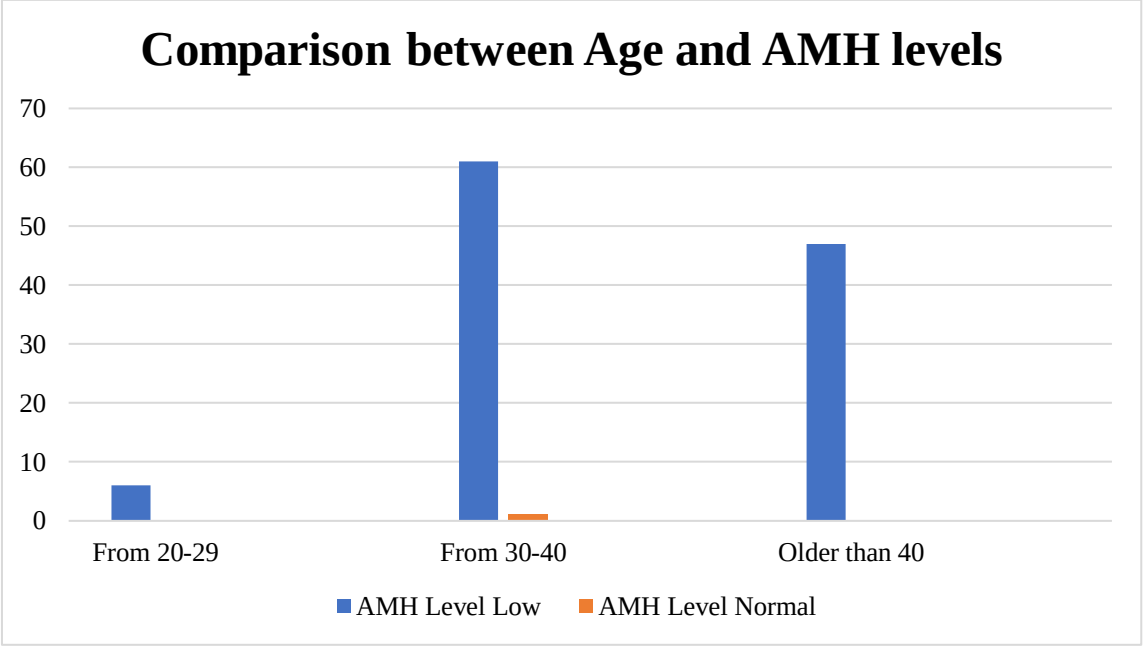

Fig. 4.7: Comparison between Age and AMH levels.

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CHAPTER FIVE

DISCUSSION

This study was done with a sample size of 115 participants, in Royal Care fertility Center in Royal Care international Hospital with the following significant**.** For the type of the infertility most of the participants have a primary type of the infertility with almost two thirds of the participants, for the FSH level majority of the patients have had a high one with more than half of them while the rest were having a normal one, for the AMH level majority of the patients have had a low type of AMH with almost all of them with an exception of one patient.

For the number of previous ICSI attempts majority of the patients have had a zero one with more than two-thirds of the participates, for the outcome of the previous ICSI 107 of the participants have said it's a zero with more than three-thirds, mostly of the participants said that they used HMG for the protocol of the ovarian stimulation with almost three-thirds of them, while for the number of the follicles produced more than four-quarters have said that its between 1-3 follicles.

For the estradiol level on day of the HCG with more than two-thirds had a level of 200-599, also showed that almost three-thirds had a level of 1-3 for the number of the oocytes, regarding the number of embryos introduced, it showed that majority of the patients have had a zero number, for the outcome of the ICSI it showed that almost two-quarters of the participants did not get pregnant.

For the comparison between the infertility and the FSH level: it showed that majority of the patients with the type of infertility as a primary has a high FSH level, which was significant (P Value 0.094), while for the secondary they had a normal one, Which was not significant (0.395), For the comparison between the infertility type and the AMH level it showed that in the primary majority had a low one, which was not significant (P Value 0.126), while for the secondary it was also with a low one, Which was not significant (P value of 0.913), For the comparison between the infertility and the estradiol level on the day of the HCG it showed that for the primary majority of the patients have had it between 200-599 as same as the secondary type, which was not significant (P Value 0.343).

For the comparison between the Age and the FSH level it showed that majority of the patients have a high and normal one for the ages of 20-29 while for the ages of 30-40 years old it was high then normal, for the patients older than 40 years old it was the same for both the high FSH level and the normal one, Which was not significant (P Value of 0.913), For the comparison between the age and the AMH for the age group from 20-29 years old it was having a low AMH, while for the ages between 30-40 years old it was having a low as same as whom are older than 40 years old, which was not significant (P Value of 0.650), regarding the AMH and the number of follicles, for the Low AMH it showed that majority of the patients have had a 1-3 number of follicles, for the normal one only one patient has had a 1-3 as a number of follicles, Which was not significant (P Value of 0.905), For the comparison between the AMH Level and the number of oocytes retrieved it showed for the low AMH level with a majority of the patients have had a number of 1-3 as same as high with one patient having it as a 1-3 number of oocytes, Which was significant (P Value of 0.093). For the Comparison between the AMH level and the number of embryos introduced majority of the patients have had a low AMH with a zero number of embryos, while for the normal one it was only one patient with a one embryo, which was significant (P Value of 0.00). For the Comparison between the AMH level and the outcome of ICSI it showed for the low result of the AMH majority of the patients have had not been pregnant, while for the normal AMH level it showed that only one patienthad a not pregnant result, which was significant (P Value of 0.01).

In a study conducted in the Department of obstetrics and gynaecology, Roskide Hospital, University of Copenhagen, in the year 2012, Studying the role of AMH in female's fertility and infertility. It was concluded that AMH in addition to other endocrine markers are frail indicators to withhold IVF treatment and it is not practical financially to suggest that a woman should undergo IVF treatment based exclusively on low AMH levels. However, AMH levels along with other factors such as age could be used as a pre-treatment counselling reference.[5] Moreover, increasing oocyte yield is based on AMH levels in poor responders is not clear as not enough studies have been done supporting the strategy and the published results on the topic have been ambiguous.Which disagrees with my study that have showed that majority of the patients have had a low AMH level and in comparison, with the ICSI and the age of a patient it showed significance and had a big difference in the numbers specially if low AMH levels, meaning that AMH levels has an impact on the outcome of an ICSI Trial.

In a study by Jenny A Visser et al, it was concluded that the examining ofovarian reserve is predominantly important in the IVF clinic, AMH was concluded to be a beneficial prognostic of poor response as numerous cases of subfertility is due to adjournment of childbearing. Nevertheless, this study determined that to obtain knowledge on whether serum AMH levels has prognostic value much more prospective studies in normal population are required to provide solid evidence of this concept. [1]This further supports my results which confirmedthe relationship between AMH and oocyte retrieval rateas being significant. Moreover, this further supports the idea of making AMH a gold standard bio marker for assessing ovarian reserve and predicting ovarian response.[18]

Concerning AMH levels according to age, AMH exhibits a progressive trend that becomes stable towards 25 years of age and then starts declining till menopause.[19]. The three age groups tested for in my study they had a P Value of 0.650**.**AMH insignificance in relation to age in my findings confirms the variation when different age groups are tested for AMH. Therefore, this is an effective tool to gauge the possible oocyte yield for patients undergoing treatment with assisted reproductive technologies and gauge response in women undergoing fertility preservation.

Furthermore, the conclusions of Tayeb et alare strengthened by the low AMH levels seen in 114 infertile women in this investigation. Their research, which was carried out at a different Khartoum fertility center, revealed a substantial drop in AMH serum concentration in infertile women as compared to control women who were fertile (p=0.00).[20]

The discordance between AMH and FSH levels found in my results complements the conclusion of Benjamin et al[13] that false reassurance arises in part because the reduction in AMH occurs before the rise in FSH. Moreover, relying soley on FSH and oestradiol will result in the same false reassurance.

The relationship between FSH and age showed a positive weak significance in a study performed by E. Gafar Abbas et al at the Ribat National university, Khartoum.[21] In my study the relationship between FSH and age showed no significance with P value of 0.913. This might be due to the increased age in this study's age distribution. However, The increase in FSH level in women after 30s is a normal physiological phenomena due to depletion of the ovarian reserve and a response from the pituitary gland.

Finally, the limitations of this study included the sample size although all the files from the time frame were included some of data from 2020 from march to may was not available due to the national lockdown which was ongoing in that period. Furthermore, other data such as ethnicity, including female of younger ages and BMI would have been of even more value but data was also not available. In addition to this the time frame set for this study did not allow for more centers to be included as well as thelimitation of access to other private clinics. Finally, females tend to seek fertility treatments in an older age due to different reasons making it harder to assess the objectives of this study in all ages.

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CHAPTER SIX

CONCLUSIONS

In conclusion 115 couples presented to the Fertility Centre of Royal care international hospital in the study period. Respectively 58% had primary infertility and 42% had secondary infertility. The important variables taken into perspective were Age, the type of infertility, FSH levels, AMH levels, Estradiol level on day of HCG, number of previous ICSI attempts and the outcome ICSI if oocytes were retrieved. In summary the study found no correlation between age and AMH, and no correlation between AMH, FSH and estradiol levels. On the other hand, a strong correlation between AMH and number of oocytes produced. Moreover, a strong correlation between AMH and success rates of ICSI was established. As so concurring with international studies in addition of being a strong indicator of premature ovarian failure.

A. Recommendations

In future studies more centers and patients should be included. A wider study sample would aid in a more thorough evaluation of the different evaluations and assessments. I would also recommend the importance of raising awareness about infertility specially in a younger age, who would have a better prognosis and a variety of treatment options. The inclusion of all parameters such as age, AMH, FSH, LH, estradiol, BMI, and AFC is of great importance for future studies to give greater depth of their value in different topics such as ovarian insufficiency and in poor responders. I also recommend the standardization of AMH measuring techniques around the world or having a conversion technique to systematize the value of AMH and therefore, help in comparing its value around the world.

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ANNEX 1

I/weconfirm thatI/we shall control and be actively engaged in the day-to-day management and conduct of the study and be responsible for all occurrences including safety of persons engaged in the project and the proper use of laboratory animals.

Signature of Project Supervisor **Date**

UMST UNIVERSITY

جامعة العلوم الطبية والتكنولوجيا
UMST University of Medical Sciences & Technology
P. O. Box 12810,Khartoum, Sudan

T. +249 183 228614
F. +249 183 224799
E. administration.office@umst-edu.sd
W. www.umst-edu.sd

I confirm that the project outline above has not already been or is currently being submitted for any other qualifications in this or other University of Institution.

Signature of Research Methodology Coordinator **Date**

(*For office use only*)

Project Number:	**Date of Submission:**

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ANNEX 2

Data collection sheet for Prevalence of female infertility based on Anti -Müllerian hormone level in Royal Care hospital, Khartoum 2020-2021

DATE:

1) Age: a. □<20 b. □ 20-29 c. □ 30-40 d.□>40
2) Type of infertility: a.□ Primary b.□ Secondary
3) FSH level: a.□ Normal b.□ Low c.□ High
4) AMH level: a.□ Normal b.□ Low c.□ High
5)No. of previous ICSI attempts: a.□ 0 b.□ 1 c.□ 2 d.□ 3 e.□ 4 f.□5

- **Outcome of previous ICSI: a.□-ve b.□ 1 c.□ 2 d.□ 3 e.□ 4 f.□5 +ve**

6) Protocol for ovarian stimulation: a.□ FSH b.□ HMG c.□ Natural cycle

7) No. of follicles produced: a.□ 1-3 b.□ 4-6 c.□ 7-10 d.□>10
8) Estradiol level on day of HCG: a.□<200 b.□ 200-599 c.□ 600-1000 d.□>1000
9) No. of oocytes retrieved: a.□ 0 b.□ 1-3 c.□ 4-6 d.□ 7-10 e.□>10
10) No. of embryos introduced: a.□ 0 b.□ 1 c.□ 2 d.□ 3 e.□ 4 f.□ 5
11)Outcome of ICSI: a.□ Pregnant b.□ Not Pregnant