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Observational Study on Effect of Age on Number of Oocyte Retrieval when Human Menopausal Gonadotropins is being given in Women Undergoing IVF

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Abstract:- Assisted reproductive technologies, specifically in vitro fertilization (IVF), have revolutionized fertility treatments, offering hope to couples facing challenges in conceiving naturally. Within the context of IVF, Human Menopausal Gonadotropin (hMG) plays a crucial role in ovarian stimulation, promoting the development of multiple follicles and increasing the likelihood of successful oocyte retrieval. However, the impact of age on the ovarian response to hMG stimulation is well-documented, with aging women often exhibiting altered patterns of follicular growth and hormonal dynamics. Advanced maternal age is associated with a decline in ovarian reserve and diminished oocyte quality, potentially influencing the outcomes of IVF cycles. This study aims to fill this knowledge gap by meticulously investigating how age influences ovarian response during IVF cycles with hMG, providing valuable insights to inform personalized and optimized fertility treatment protocols for women across different age groups. Retrospective analysis of 113 women aged 26-45 undergoing IVF with hMG at selected site in period of 3 months. Extracting relevant data from patient records, including age, baseline hormones, stimulation details, AFC count, and oocyte retrieval. The obtained data was analysed by descriptive analysis (ANOVA) using Ms excel. An overview of the oocyte yield for every age group may be seen in the average total oocyte count. According to the data, the mean number gradually decreases with age, which is consistent with aging-related decreases in follicular response and ovarian reserve. The study shows that the 26-30 age group had the highest number of oocytes while oocyte Numbers in the age groups of 31–35, 36–40, and 41–45 indicate a consistent age-related drop in the total oocyte retrieval count. This reduction occurs as a result of aging-related declines in ovarian reserve and follicular quality, which lessen the ovarian response to stimulation during IVF treatment.

Keywords:- *IVF, Human Menopausal Gonadotropin (hMG), Oocyte, Age, AFC, Infertility.*

I. INTRODUCTION

➢ *Introduction of IVF*

IVF, or in vitro fertilization, has completely changed the field of reproductive medicine and given hope to infertile couples. Since its inception in the latter half of the 20th century, in vitro fertilization (IVF) has developed into an advanced and extensively utilized assisted reproductive technology (ART).In vitro fertilization (IVF) is the process of fertilizing an egg with sperm outside of the body and then transferring the resultant embryo into the uterus. Through this complex procedure, individuals and couples can overcome a variety of hurdles connected to fertility, such as problems with the function of the fallopian tube, male factor infertility, endometriosis, and infertility that is unexplained.[1]

The first successful IVF baby was born in 1978 when Louise Brown became the first to be conceived through IVF. IVF has since become a widely used and constantly improving procedure, helping to conceive millions of babies around the world. IVF is a complex process that includes several key stages:

- Ovarian stimulation
- Egg retrieval
- Fertilization in a lab setting
- Embryo culture
- Transfer of the embryo/s into the uterus

Not only has IVF become a common treatment for infertility, but it has also helped advance other reproductive technologies. The success rate of IVF has steadily increased over time, and research and technological advances continue to improve its effectiveness. A thorough understanding of IVF, and its constant improvement, highlights its importance as a revolutionary tool in the quest for parenthood for those struggling to conceive naturally.[2]

Age-related decline in fertility is well-documented and is mainly due to changes in oocyte quantity and quality. As a woman ages, her ovarian reserve, which is a pool of eggs that can be fertilized, decreases. There is also an increased risk of chromosome abnormalities in the egg, which can lead to decreased fertility and an increased risk of abortion. A comprehensive review of the effects of age-related decline on

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fertility in women found that fertility decreases in women in their late 20s and accelerates in women in their mid to late 30s and increases in women after 40 years of age. Women who attempt to conceive naturally have a lower chance of spontaneous pregnancy, while those who attempt natural conception have a higher chance of infertility and higher risk of pregnancy complications.[3]

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) can both be found in postmenopausal women's urine, which is used to make HMG. The growth and maturation of ovarian follicles are stimulated by FSH, whereas ovulation is initiated by LH. When FSH and LH are combined in hMG, ovarian stimulation in IVF is supported by a more complete hormonal response.[4]

Women are given hMG to induce controlled ovarian hyperstimulation during IVF treatment. To do this, more follicles—each containing an egg—must be recruited and allowed to grow. By simulating the natural hormonal environment, the exogenous FSH and LH in hMG promote the growth and maturation of multiple follicles, thereby increasing the yield of mature eggs that can be harvested during the IVF procedure. HMG is a preparation derived from the urine of postmenopausal women, containing both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH is essential for stimulating the growth and maturation of ovarian follicles, while LH triggers ovulation. The combination of FSH and LH in hMG ensures a more comprehensive hormonal support during ovarian stimulation in IVF.[4]

During IVF treatment, hMG is administered to women to induce controlled ovarian hyperstimulation. This involves boosting the recruitment and development of multiple follicles, each containing an egg. The exogenous FSH and LH in hMG simulate the natural hormonal milieu, supporting the growth and maturation of multiple follicles, ultimately increasing the yield of mature eggs available for retrieval during the IVF process. The incorporation of hMG in IVF protocols addresses the challenge of inadequate follicular development, especially in women with diminished ovarian reserve or those who exhibit suboptimal response to fertility medications. By providing exogenous FSH and LH, hMG augments the natural hormonal milieu, optimizing the chances of obtaining a sufficient number of mature eggs for fertilization. The significance of hMG is further emphasized in cases where a more robust ovarian response is desired to enhance the likelihood of a successful IVF cycle. The tailored use of hMG allows clinicians to individualize treatment protocols, ensuring optimal ovarian stimulation for diverse patient profiles.[5]

- *Count of Oocytes and Aging:*

Oocyte count is crucial for in vitro fertilization (IVF), especially in light of the recognized link between declining ovarian reserve and increasing mother age. IVF treatment success rates are impacted by the amount and quality of oocytes declining with age in women. This introduction highlights the crucial role that oocyte count plays as a major predictor of IVF outcomes and examines its important significance in the setting of advancing age. The basis for effective IVF outcomes is oocytes, or eggs. When ovaries age, there is a decrease in the quantity of oocytes that can be extracted and a higher chance of chromosomal abnormalities. An important obstacle in the process of achieving fertility is the age-related decrease in oocyte number and quality.[6]

A large body of research, including a thorough meta-analysis has shown a direct correlation between aging mothers and a reduction in ovarian reserve, which is manifested as a lower oocyte count. The quantity of oocytes accessible for IVF treatments declines as women get closer to their late 30s and beyond, which has a direct impact on the likelihood of successful fertilization and embryo development. In IVF cycles, there is a positive correlation between a larger oocyte yield and a better likelihood of a live birth. On the other hand, decreased success rates are linked to oocyte count declines with age, highlighting the crucial role oocyte quantity plays in determining the overall outcome of IVF therapies.[6]

All of a female's oocytes are present at birth, even if they may be in an arrested state for a long time. Oocytes lose quality or experience programmed cell death as mothers age. Optimal female fertility, however, lasts until about the age of thirty before precipitously declining after that. Consequently, the chance of infertility has grown due to the tendency over the past 30 years of delaying childbearing. Furthermore, the total number of oocytes in ordinary women of reproductive age hits a threshold of about 25,000 at the age of 37–38 years, after which the numbers rapidly decrease.[7]

The effects of aging on women's fertility are complex and impacted by a number of variables, including ovarian reserve alterations. Antral Follicle Count (AFC) and Anti-Müllerian Hormone (AMH) are recognized indicators that are used to evaluate ovaria reserve and offer important information about a woman's capacity for reproduction. This introduction explores the relevance of AFC count as a predictor of ovarian reserve and fertility, highlighting its significance as women age. Clinical professionals can assess a woman's potential for conception by using the decrease in AFC count, which acts as an early warning sign of the ovarian reserve being depleted. When it comes to choosing the best time to conceive or investigate possibilities for fertility preservation, this information becomes quite helpful for women who are thinking about starting a family. Comprehending the correlation between advancing age and decreasing AFC count facilitates customized fertility advice and the creation of optimized tactics for assisted reproductive technologies. Through AFC examinations, healthcare providers can identify how aging affects ovarian reserve and help women plan ahead for pregnancy.[8]

- *Oocyte Collection:*[9]

Oocyte retrieval, sometimes referred to as egg retrieval or oocyte harvest, is an essential step in in vitro fertilization (IVF) and other assisted reproductive technologies. Mature eggs are removed from a woman's ovaries and fertilized in a lab during the process. With a reference, below is a general rundown of the oocyte collection procedure:

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- Procedure for Oocyte Collection: Transvaginal ultrasound-guided follicular aspiration is the procedure commonly used for transvaginal oocyte harvesting. The following actions are necessary for this:
- Ovarian Stimulation: using fertility drugs to stimulate women's ovaries prior to oocyte collection. This causes the formation of many follicles, each of which contains an egg.
- Monitoring: Blood tests to determine hormone levels and transvaginal ultrasound images are used to track the development and maturity of the follicles.
- Triggering Ovulation: To cause final maturation and preparation for ovulation, a trigger shot of human chorionic gonadotropin (hCG) is given to the follicles after they reach the proper size.
- Oocyte Retrieval: The actual retrieval of oocytes occurs about 36 hours following the trigger shot. A tiny needle is injected through the vaginal wall into the ovaries under ultrasound supervision. The fluid that contains the eggs is sucked from the follicles using a needle that is connected to a suction device.
- Laboratory Processing: To identify and separate the eggs, the recovered fluid is checked right away in the lab.
- Fertilization: After the mature eggs are fertilized in a lab with sperm, the resultant embryos are cultivated for a few days and then placed into the woman's uterus.

In vitro fertilization (IVF) retrieved oocytes can be categorized according to their developmental stage and maturity. There are various stages at which oocytes can be extracted, and the sort of oocytes that are retrieved can affect how well IVF treatments work. This is a broad categorization:

- Oocytes at maturity (Metaphase II): These oocytes have undergone both meiotic divisions and are fully developed. When it comes to IVF fertilization, they are the favoured kind.
- Immature Oocytes: extracted oocytes prior to complete maturity (meiosis completion). Before fertilization, they might undergo in vitro maturation (IVM) in a lab.
- Oocytes from Germinal Vesicles (GVs): oocytes that retain the germinal vesicle, or nucleus. Prior to fertilization, these oocytes may go through in vitro maturation.
- In Metaphase I Ovum: eggs that have finished their first round of meiosis but not their second. Before fertilization, they can go through in vitro maturation to reach metaphase II.

Numerous factors affect the success of in vitro fertilization (IVF), and maximizing results requires an awareness of these elements. The following are some important variables influencing IVF success: maternal age, ovarian reserve , embryo quality , uterine factors like fibroids , sperm quality and quantity.

Depending on the particular requirements of the individual or couple, in vitro fertilization (IVF) may involve a variety of techniques and procedures. Here are a few popular IVF techniques:

- Traditional IVF: Involves causing the ovaries to release several eggs, collecting the eggs, fertilizing them in a lab with sperm, and putting the developing embryos inside the uterus.
- Injectable sperm culture (ICSI): To aid in fertilization, a single sperm is directly inserted into an egg.
- Testing for preimplantation genetics, or PGT: include checking embryos for genetic defects prior to transfer.
- FET (Transplanting Embryos): After being frozen throughout an IVF round, the embryos are thawed and inserted into the uterus.
- IVF Using a Natural Cycle: involves taking an egg that has spontaneously reached maturity and fertilizing it without ovarian stimulation.

➢ *Types of Infertility:*[10]

- Primary Infertility: The failure of a couple to conceive or give birth to a live child following at least a year of frequent, unprotected sexual activity without any prior pregnancies is known as primary infertility.
- Causes: Ovulatory problems, tubal factors, uterine abnormalities, male factor infertility, and unexplained variables are some of the causes of primary infertility.
- Infertility that occurs later on- secondary infertility: After one or more previous successful pregnancies, a couple experiences trouble conceiving or achieving a live birth. This condition is known as secondary infertility.
- Causes: Age-related declines in fertility, modifications to reproductive health, or new factors that have emerged since the previous pregnancy are some of the causes of secondary infertility, which are comparable to those of primary infertility.

➢ *Undoubtedly, the Following are some Causes of Infertility in Both Gender:*

- In male – sperm abnormalities , varicocele, hormonal imbalances
- In female – ovulatory disorders like PCOS ,tubal factors , uterine factors

Despite being typically safe, in vitro fertilization carries significant risks and difficulties. The following are a few potential complexities:

- Ovarian hyperstimulation syndrome (OHSS): Following ovarian stimulation and oocyte retrieval, this uncommon but potentially dangerous complication might manifest. Abdominal pain, bloating, nausea, vomiting, and shortness of breath are some of the signs and symptoms of OHSS.
- Bleeding or infection: During egg retrieval, there may be severe bleeding from the ovaries or damage to organs nearby, such as the bladder, colon, or blood vessels. Another uncommon but potential consequence is pelvic infection.
- Multiple pregnancies: Induced family planning (IVF) raises the risk of multiple pregnancies, which can result in issues like low birth weight and early birth

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- Birth problems: Compared to normally conceived offspring, children conceived through IVF have a slightly greater chance of birth defects.
- Miscarriage and ectopic pregnancy: Women who conceive naturally have a somewhat lower risk of miscarriage and ectopic pregnancy than do women who conceive through IVF.

Even yet, risks are extremely uncommon and can be avoided with the right advice and precautions. It is crucial to consider every potential benefit and risk of in vitro fertilization and understand how it affects mothers as they age. A successful IVF cycle should be achieved by selecting and implementing the best therapy and procedure available.

The need for the study, focuses in addressing an important component of assisted reproductive technology (ART): the interaction of ovarian response, age, and the use of Human Menopausal Gonadotropin (HMG) in in vitro fertilization (IVF). It is important to comprehend this interplay for a number of reasons:

- Optimizing treatment protocol: The results of the study can help improve IVF treatment plans, especially when it comes to adjusting ovarian stimulation tactics according to a woman's age. This is in line with the more general suggestions for customized methods of ovarian stimulation during in vitro fertilization.
- Enhancing success rates: The study can help develop techniques to increase IVF success rates by identifying age-specific changes in ovarian response to HMG, particularly in situations where unsatisfactory response, as described by the Bologna criteria, is more likely.
- Personalized assisted technology strategies : In keeping with the current trend towards targeted patient care in fertility treatment, the study's insights into age-related changes in oocyte retrieval using HMG can help design personalized assisted reproductive techniques.
- Reducing treatment risks: In line with the objective of enhancing the safety profile of IVF treatments, comprehending the age-dependent response to HMG may help lower the hazards related to ovarian hyperstimulation, multiple pregnancies, and other problems.

In conclusion, the study fills a significant knowledge vacuum about age-related differences in oocyte retrieval during IVF with HMG, offering insightful information that might help develop safer, more individualized, and more successful reproductive treatment plans. The citations offered provide credence to the ongoing attempts to improve IVF procedures for better results as well as the larger background of tailored techniques in assisted reproductive medicine.[11]

II. MATERIAL AND METHODOLOGY

➢ *Type of Study:*

The study is retrospective observational study of female patient which has undergone IVF treatment at chosen site.

➢ *Study Population:*

Prevalence Rate : study from India found that about eight percent (8%) of currently married women suffered from primary and secondary infertility.[33]

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where,

Z = statistic for a level of confidence (for level of confidence 95%, which is conventional, Z value is 1.96

p = Expected prevalence or proportion = 0.08
d = Precision (d is considered 0.05 to produce good precision and small error of estimate).

$$n = \frac{(1.96)^2\ 0.08(1-0.08)}{(0.05)^2}$$

$$n = 113$$

Total sample size: 113

All the female patient who went under IVF treatment to whom hMG was only being given for ovulation stimulation was included.

➢ *Study Procedure:*

Case sheets having all needed information of patient like age, BMI, type of fertility, past medical history, AFC, etc was randomly selected and all data were noted down under guidance of healthcare professionals.

➢ *Inclusion Criteria:*

- Women undergoing IVF treatment with antagonist protocol.
- Age between 26 - 45 Years
- Patient to whom hMG is being given
- Confirm infertility of various origin
- BMI in range of 18-25 (normal)
- Antral follicle count >3

➢ *Exclusion Criteria:*

- Age Group < 26 and > 45 Year
- Women undergoing treatment other than hMG.
- Medical risk factors like heart disease, cancer, renal disease or liver disease
- Antral follicle count <3.
- Patient undergone IVF, IVI, Ovarian stimulation in last 6 months
- Oocyte donor undergoing ovulation induction.
- Patient who has not completed dosage cycle.

➢ *Data Collection Form:*

The data collection form is divided into three parts, first part collects the patient demographics data including age, BMI, weight and height. second part consist of patient having

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which type of infertility, their past medical, medication, social and family history along with lab data. And the final part includes antral follicle count, dose of hMG, no of days of treatment, no. total oocyte retrieved which is main parameter of study.

➢ *Statistical Analysis:*

Finding Mean, Percentage, Graphical Presentation and ANOVA Test using Microsoft Excel.

III. RESULT

➢ *Distribution of Study Population on the Basis of Age Group :*

Table 1 shows that there were total 113 patients are enrolled, among this highest number of 32.7% (n=37) of the patients were under the age group of 26 - 30 Years (group 1), 30.1% (n=34) of the patients were under the age group of 31 - 35 Years (group 2), 26.5% (n=30) of the patients were under the age group of 36 – 40 Years (group 3), 10.6% (n=12) of the patients were under the age group of 41 – 45 Years (group 4).

Table 1 Distribution of the Study Population Based on Age

Age Group	Number of Patients	Percentage (%)
26 – 30 (group 1)	37	32.74
31 – 35 (group 2)	34	30.09
36 – 40 (group 3)	30	26.55
41 – 45 (group 4)	12	10.62
Total	113	100

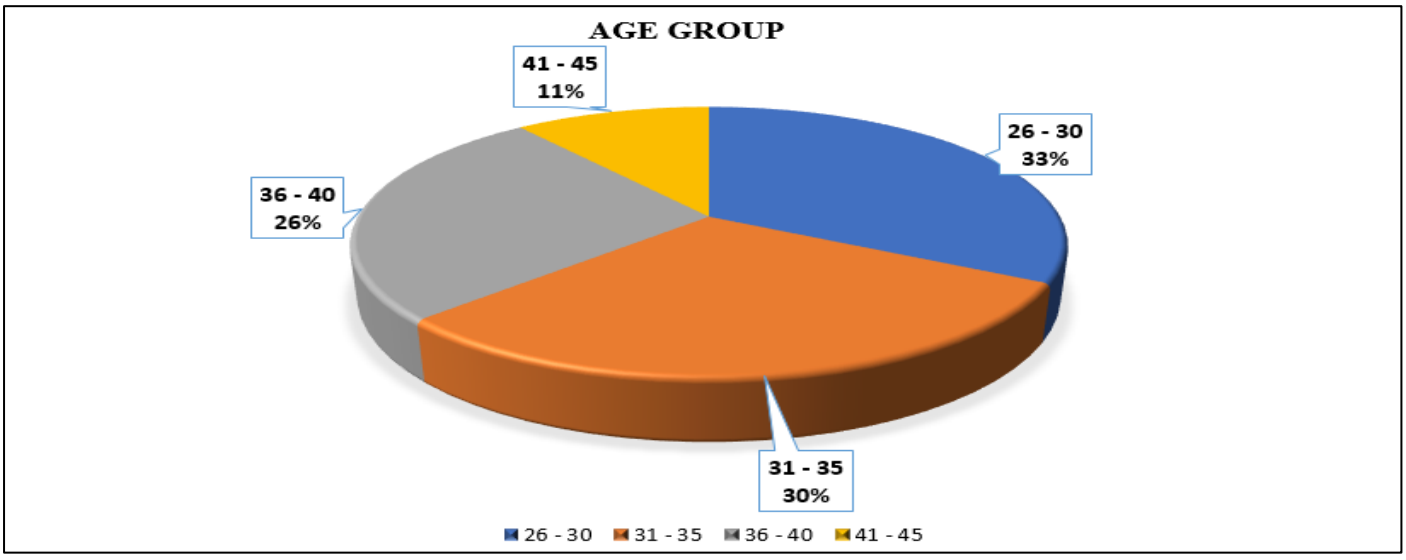

Fig 1 Distribution of Study Population Based on Age Group

➢ *Assessment of Total Oocyte Retrieval Count in Different Age Group :*

There is total 1157 total oocyte retrieval count in all the age group. There is highest total oocyte retrieval count in group of 26 – 30 years (group 1), total oocyte count in (group 1) 26-30 years age group is 443 (n=37). In (group 2) group of 31-35 years total oocyte retrieval count is 400 (n=34). In (group 3) group of 36-40 years total oocyte retrieval count is 254 (n=30). There is lowest total oocyte retrieval count in (group 4) group of 41 – 45 years, total oocyte count in 41-45 years age group (group 4) is 60 (n=12). Total oocyte count, frequency of distribution and mean total oocyte count is shown in Table 2. While graphical representation of total oocyte retrieval vs age group is shown below in figure 2 and figure 3.

Table 2 Total Oocyte Retrieval Count in Different Age Group

	Total Oocyte retrieval in 26 - 30 Age Group (group - 1)	Total Oocyte retrieval in 31 - 35 Age Group (group - 2)	Total Oocyte retrieval in 36 - 40 Age Group (group - 3)	Total Oocyte retrieval in 41 - 45 Age Group (group - 4)
SUB TOTAL	443	400	254	60
TOTAL	1157			
n	37	34	30	12
MEAN TOTAL OOCYTE RETRIEVAL COUNT	11.97	11.76	8.47	5
PERCENTAGE	38.29 %	34.57 %	21.95 %	5.19

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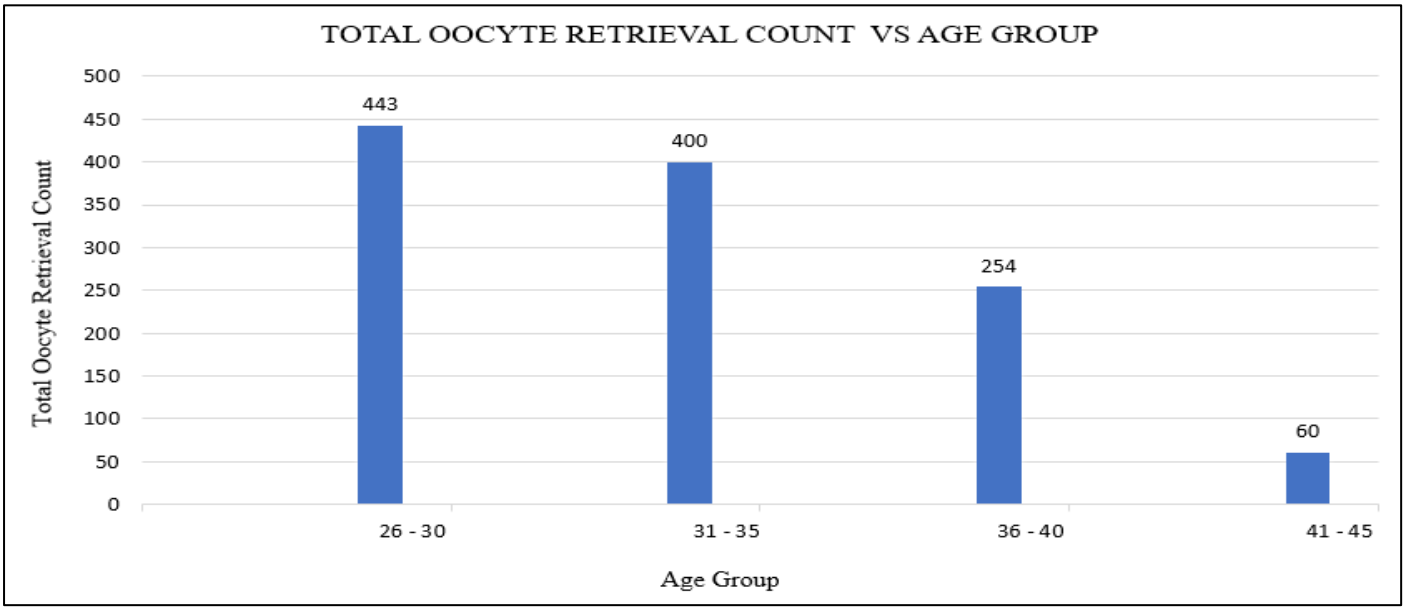

Fig 2 Total Oocyte Retrieval Count vs Age Group

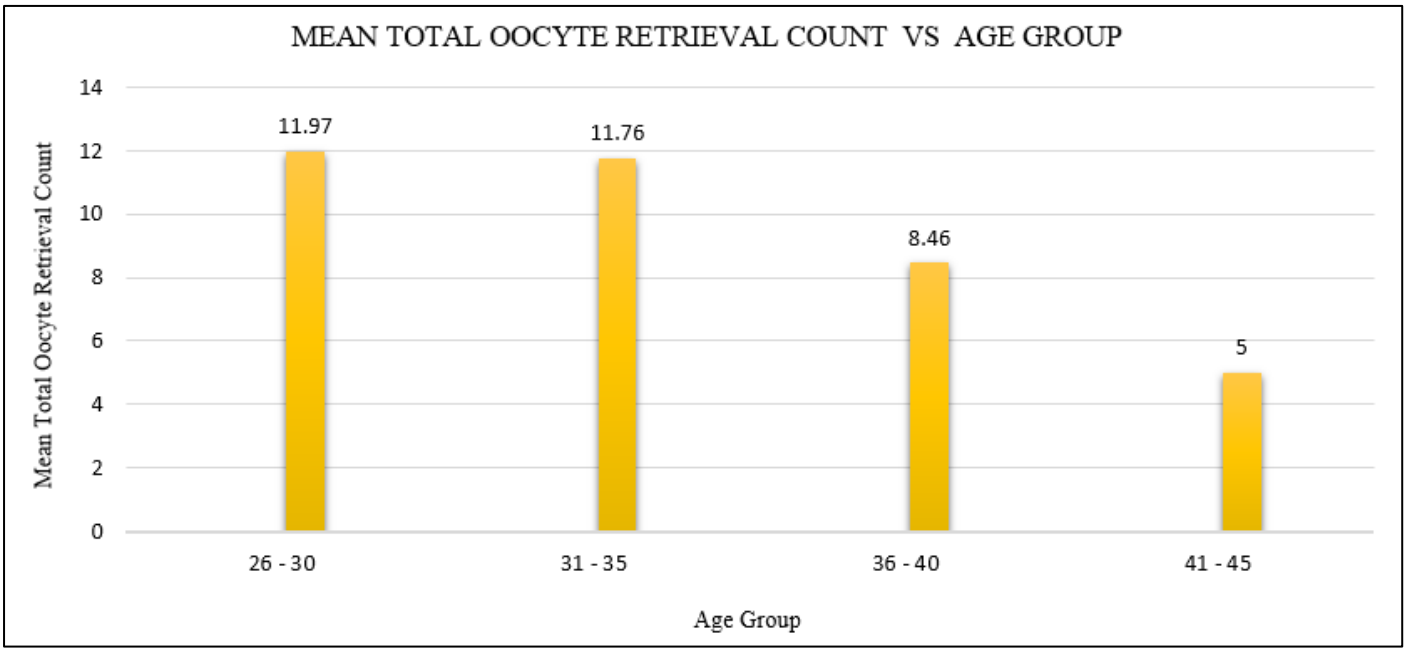

Fig 3 Mean Total Oocyte Retrieval Count vs Age Group

➢ *Statistical Analysis for Mean Total Oocyte Retrieval count in Different Age Group:*

One Way ANOVA Test : One way ANOVA test is used as hypothesis testing tool to determine if there is significant difference between the means of total oocyte retrieval count in four age group.

Table 3 Summery of Total Oocyte Retrieval Count in Different Age Group

Groups	Number of patients (n)	Sum of Oocyte retrieval count	Average	Variance
26-30 Age group (group 1)	37	443	11.97	44.03
31-35 Age group (group 2)	34	400	11.76	46.06
36-40 Age group (group 3)	30	254	8.47	47.36
41-45 Age group (group 4)	12	60	5	10

Table 4 One way ANOVA Test for mean Total Oocyte Retrieval Count in Different Age Group

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	613.99	3	204.66	4.86	0.0033	2.69
Within Groups	4588.56	109	42.10			
Total	5202.548673	112				

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Null hypothesis = There is no significant difference between mean of total oocyte retrieval count in different age group.

Alternative hypothesis = There is significant difference between mean of total oocyte retrieval count in different age group.

p-value = 0.05

If calculated *p-value* is greater than 0.05 that indicates accept null hypothesis, and calculated *p-value* is less than or equal to 0.05 that indicates reject null hypothesis.

In this table calculated *p-value* is 0.0033 (< 0.05). Therefore, we reject null hypothesis and accept alternative hypothesis. Hence there is significant difference between mean of total oocyte retrieval in different age group.

For the determination of which groups are significantly different from another we will perform Post-hoc Test such as Bonferroni Test.

Table 5 Corrected alpha (α) value for oocyte retrieval count

Test	Alpha (P-value)
ANOVA	0.05
Post-hoc test (Bonferroni corrected)	0.0083

Table 6 Post-Hoc Test for oocyte retrieval count

Group	P-value (T test)	Significant ?
group 1 vs group 2	0.90	No
group 1 vs group 3	0.038	No
group 1 vs group 4	0.001	Yes
group 2 vs group 3	0.058	No
group 2 vs group 4	0.002	Yes
group 3 vs group 4	0.103	No

Null hypothesis = There is no significant difference between mean of total oocyte retrieval count in different age group.

Alternative hypothesis = There is significant difference between mean of total oocyte retrieval count in different age group.

In Table 5 POST-HOC Test corrected *p-value* is 0.0083

If calculated *p-value* is greater than 0.0083 that indicates accept null hypothesis, and calculated *p-value* is less than or equal to 0.0083 that indicates reject null hypothesis.

Hence there is no significant difference in mean total oocyte retrieval in Group 1 (26-30 Year Age group), Group 2 (31-35 Year Age group), and Group 3 (36-40 Year Age group). There is significant difference in Group 4 (41-45 Year Age group).

➢ *Assessment of Total AFC Count in Different Age Group:*

There is total 1421 total AFC count in all the age group. There is highest AFC count in group of 26 – 30 years, total AFC count in 26-30 years age group is 537 (n=37). In group of 31-35 years total AFC count is 502 (n=34). In group of 36-40 years total AFC count is 296 (n=30). In group of 41-45 years total AFC count is 86 (n=12). Total AFC count, frequency of distribution and mean total AFC count is shown in table 7. While graphical representation of total AFC count vs age group is shown below in figure 4 and figure 5.

Table 7 Total AFC count in different Age group

	Age group 25-30 (group – 1)	Age group 31-35 (group – 2)	Age group 36-40 (group – 3)	Age group 41-45 (group – 4)
Sub Total	537	502	296	86
n	37	34	30	12
Total	1421			
Mean	14.51	14.76	9.87	7.17
Percentage %	37.79 %	35.33 %	20.83 %	6.05 %

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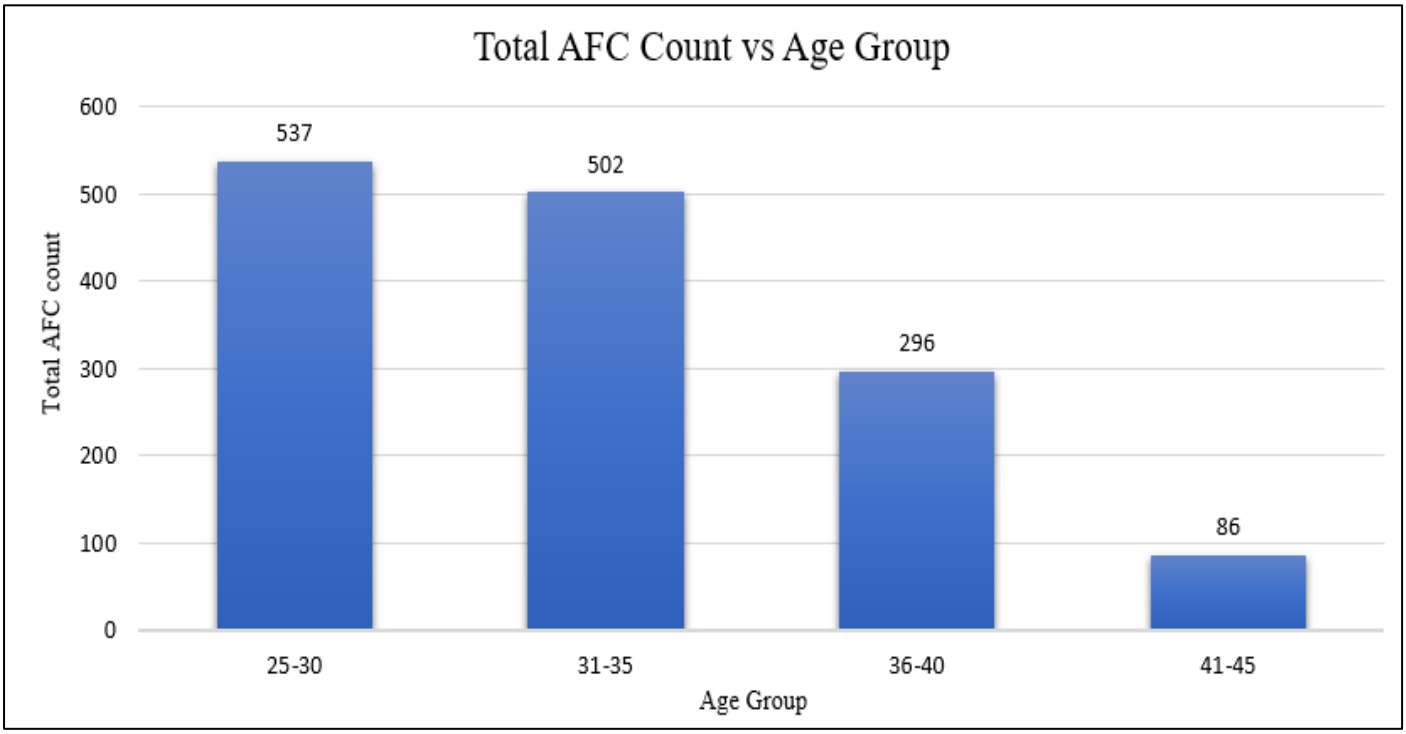

Fig 4 Total AFC count vs Age group

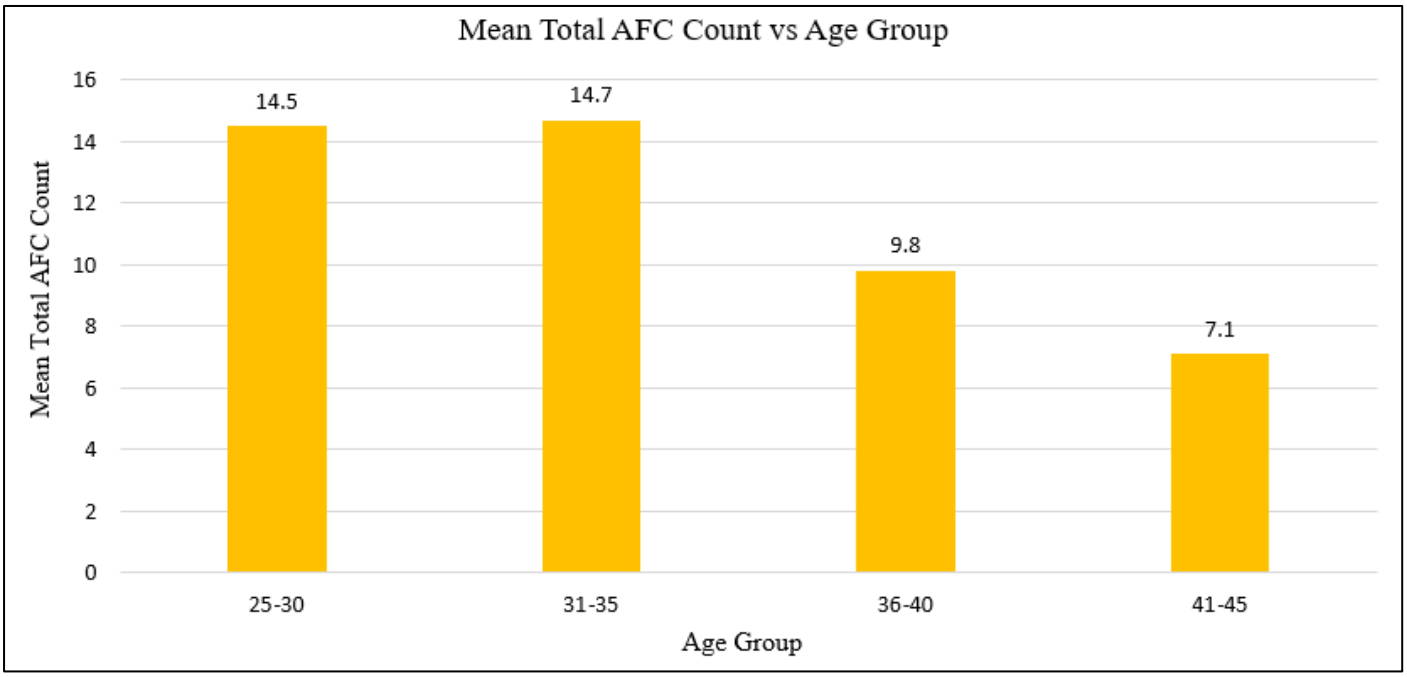

Fig 5 Mean Total AFC Count vs Age Group

➢ *Statistical Analysis for mean AFC Count in Different Age Group:*

One Way ANOVA Test : One way ANOVA test is used as hypothesis testing tool to determine if there is significant difference between the means of AFC in four age group.

Table 8 Summery of AFC Count in Different Age Group

Groups	Number of patient (n)	Sum of AFC count	Average	Variance
Age group 25-30 (group - 1)	37	537	14.51	43.77
Age group 31-35 (group - 2)	34	502	14.76	39.44
Age group 36-40 (group - 3)	30	296	9.87	35.71
Age group 41-45 (group - 4)	12	86	7.17	11.56

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Table 9 One way ANOVA Test for mean AFC Count in Different Age Group

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	873.12	3	291.04	7.85	0.000086	2.69
Within Groups	4039.99	109	37.06			
Total	4913.11	112				

Null hypothesis = There is no significant difference between mean of AFC count in different age group.

Alternative hypothesis = There is significant difference between mean of AFC count in different age group.

p-value = 0.05

If calculated *p-value* is greater than 0.05 that indicates accept null hypothesis, and calculated *p-value* is less than or equal to 0.05 that indicates reject null hypothesis.

In this table calculated *p-value* is 0.000086 (< 0.05). Therefore, we Reject null hypothesis and Accept alternative hypothesis. Hence there is significant difference between mean of AFC count in different age group.

For the determination of which groups are significantly different from another we will perform Post-hoc Test such as Bonferroni Test.

Table 10 Corrected alpha (α) value for AFC count

Test	Alpha
ANOVA	0.05
Post-hoc test (Bonferroni corrected)	0.0083

Table 11 Post-Hoc Test for AFC count

Group	P-value (T test)	Significant ?
group 1 vs group 2	0.87	No
group 1 vs group 3	0.004	Yes
group 1 vs group 4	0.0006	Yes
group 2 vs group 3	0.0022	Yes
group 2 vs group 4	0.0002	Yes
group 3 vs group 4	0.1504	No

Null hypothesis = There is no significant difference between mean of total oocyte retrieval count in different age group.

Alternative hypothesis = There is significant difference between mean of total oocyte retrieval count in different age group.

In Table 10 POST-HOC Test corrected *p-value* is 0.0083

If calculated *p-value* is greater than 0.0083 that indicates accept null hypothesis, and calculated *p-value* is less than or equal to 0.0083 that indicates reject null hypothesis.

Hence there is no significant difference of mean AFC count in Group 1 (26-30 Year Age group) and Group 2 (31-35 Year Age group). There is no significant difference of mean AFC count in Group 3 (36-40 Year Age group) and Group 4 (41-45 Year Age group). But there is significant difference of Mean AFC count Group 1 (26-30 Year Age group) and Group 3 (36-40 Year Age group), Group 1 (26-30 Year Age group) and Group 4 (41-45 Year Age group), Group 2 (31-35 Year Age group) and Group 3 (36-40 Year Age group), Group 2 (31-35 Year Age group) and Group 4 (41-45 Year Age group).

➢ *Assessment of Total dose of hMG in Different Age Group:*

The total dose of hMG is 476250 IU in all the age group. There is highest total hMG dose in group of 26 – 30 years, total dose of hMG in 26-30 years age group is 156150 IU (n=37). In group of 31-35 years total hMG dose is 138150 IU (n=34). In group of 36-40 years total hMG dose is 129450 IU (n=30). In group of 41-45 years total hMG dose is 52500 IU (n=12). Total hMG dose, frequency of distribution and mean total hMG dose is shown in Table. While graphical representation of total dose vs age group is shown below in graph.

Table 12 Total hMG dose (IU) in Different Age Group

	Age group 25-30 (group – 1)	Age group 31-35 (group – 2)	Age group 36-40 (group – 3)	Age group 41-45 (group – 4)
Sub Total	156150	138150	129450	52500
n	37	34	30	12
Total	476250			
Mean	4220.27	4063.24	4315	4375
Percentage	32.79 %	29.01 %	27.18 %	11.02 %

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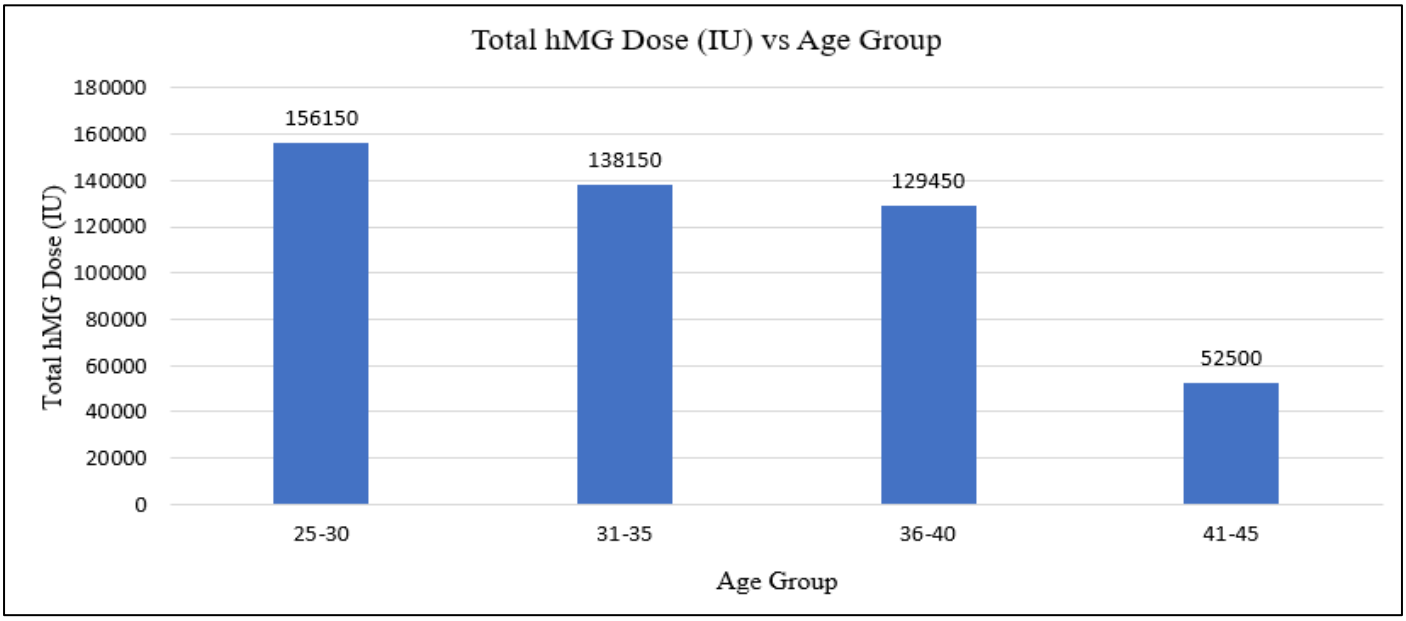

Fig 6 Total hMG Dose (IU) vs Age Group

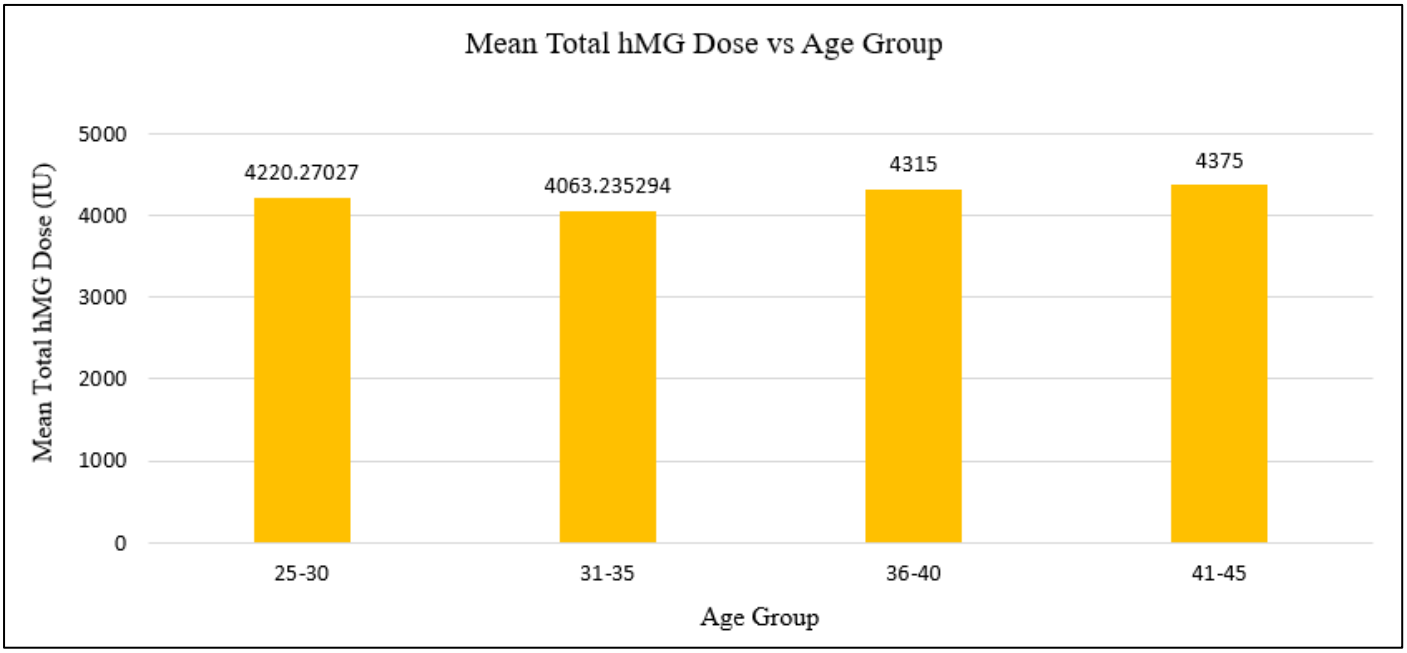

Fig 7 Mean Total hMG Dose vs Age Group

➢ *Statistical Analysis for mean hMG Dose in Different Age Group:*

One Way ANOVA Test : One way ANOVA test is used as hypothesis testing tool to determine if there is significant difference between the mean dose of hMG in four age group.

Table 13 Summery of hMG dose in Different Age Group

Groups	Number of patient (n)	Sum of Dose	Average	Variance
Age group 25-30 (group – 1)	37	156150	4220.27	316452.7
Age group 31-35 (group – 1)	34	138150	4063.24	825501.3
Age group 36-40 (group – 1)	30	129450	4315	322267.2
Age group 41-45 (group – 1)	12	52500	4375	195681.8

Table 14 One way ANOVA Test for mean hMG dose in Different Age Group

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1391316	3	463771.9	1.008359	0.39	2.69
Within Groups	50132091	109	459927.4			
Total	51523407	112				

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Null hypothesis = There is no significant difference between mean of hMG dose in different age group.

Alternative hypothesis = There is significant difference between mean of hMG dose in different age group.

p-value = 0.05

If calculated *p-value* is greater than 0.05 that indicates accept null hypothesis, and calculated *p-value* is less than or equal to 0.05 that indicates reject null hypothesis.

In this table calculated *p-value* is 0.39 (> 0.05). Therefore, we Accept null hypothesis and Reject alternative hypothesis. Hence there is no significant difference between mean of hMG dose in different age group.

IV. DISCUSSION

The study likely divided participants into different age groups to examine how ovarian response differed across these categories. This analysis would provide detailed insight into how age affects ovarian function and response to hMG stimulation. This can reveal critical thresholds or tipping points where the decline in ovarian reserve accelerates, leading to a marked reduction in oocyte retrieval. By identifying age-specific trends in ovarian response, clinicians can more accurately tailor treatment protocols to optimize outcomes for women at different stages of reproductive aging.

Our study was done on 113 patients that were all female and resided under age group of 26 to 45 years. In which some patient had primary (60.20) % while other had secondary (39.80) % infertility. Out of all patients there were cases of presence of male partner infertility (in 31 patients). All our patient was given human menopausal gonadotropin treatment for defined period of time for ovarian stimulation, so at the time of ovum pickup, oocyte retrieval was measured which resulted in Total Oocyte retrieval counts in different Age group (mean %) as following

Age group 26-30 year had 38.28867761 % then group 31 – 35 year had 34.5721694 % group 36-40 year had 21.95332757 and lastly group 41-45 year had 5.185825411 % of mean total oocyte retrieval respectively. The results show a clear trend in the total number of oocytes in different age groups. The largest part was observed in the age group of 26-30 years and the least in the age group of 41-45 years.

Average total oocyte count and distribution: The average total oocyte count is a summary of the oocyte yield for each age group. The analysis shows a gradual decline in mean number with age, reflecting age-related declines in ovarian reserve and follicular response. In addition, the distribution of oocyte counts between age groups describes the relative contribution of each group to the total oocyte count, which further emphasizes the differences in ovarian function between different age groups.

The study shows that the 26-30 age group had the highest number of oocytes with a total of 443 oocytes. This finding is consistent with established knowledge in reproductive medicine, where women in their 20s and 30s typically have the highest ovarian reserve and follicular sensitivity to stimulation. Strong ovarian function in this age group contributes to increasing the number of eggs collected during IVF procedures.

The numbers in the age categories of 31–35, 36–40, and 41–45 show that the overall oocyte retrieval count steadily declines with age. This decrease is a result of follicular quality and ovarian reserve declining with age, which reduces the ovarian response to stimulation during IVF therapy. When compared to younger age groups, women in their late 30s and early 40s have a significant decrease in their oocyte retrieval count, which suggests that they have less ovarian reserve and less potential for conception.

Statistical analysis with one-way ANOVA followed by Bonferroni's corrected post-hoc test provides valuable information on differences in mean total oocyte count by age group.

A one-way ANOVA test examines whether there is a significant difference in the mean total number of oocytes among the four age groups. The calculated p-value (0.0033) is less than the significance level ($\alpha = 0.05$) indicating that there is a statistically significant difference in mean total production of oocytes between age groups.

Bonferroni-corrected post-hoc test: post-hoc test, specifically the Bonferroni-corrected pairwise comparison, further examines which specific age groups have significant differences in the mean total number of oocytes. The corrected significance level ($\alpha = 0.0083$) adjusts for multiple comparisons to reduce the risk of type I error.

Bonferroni-corrected post-hoc test results show that the difference between the mean total numbers of oocytes per 1 is not significant .group (26-30 years), between the 2nd group (31-35 years) and the 3rd group (36-40 years). However, there is a significant difference in the average number of egg retrievals for group 4 (aged 41-45) compared to other age groups.

Evaluation of total antral follicle count (AFC) in different age groups provides valuable information about ovarian reserve and follicle density in women undergoing fertility evaluation. Mean total AFC % in different age group were found as follows: age group 26 to 30 had 37.79028853%, age group 31 to 35 had 35.32723434%, group 36 to 40 had 20.83040113% and group 41 to 45 had 6.052076003%.

The data show a clear trend toward a decline in AFC with age. AFCs are most abundant in the youngest age group (26-30 years) with a total of 537, while the least in the oldest age group (41-45 years) with only 86 follicles. This finding is consistent with established knowledge in reproductive medicine, which recognizes a gradual decline in ovarian reserve and follicle density as women age.

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Statistical analysis with a one-way ANOVA test followed by Bonferroni's corrected post-hoc test provides significant insight into differences in mean antral-follicle count (AFC) between age groups.

A one-way ANOVA test examines whether there is a significant difference in the mean AFC value of the four age groups. The calculated p-value (0.000086) is less than the significance level ($\alpha = 0.05$) indicating that there is a statistically significant difference in mean AFC between age groups.

Post-hoc a post hoc test, specifically a Bonferroni-corrected pairwise comparison, further examines where there are significant differences in mean AFC within specific age groups. The adjusted significance level ($\alpha = 0.0083$) adjusts for multiple comparisons to reduce the risk of Type I error.

Bonferroni-corrected post-hoc test results show that there is no significant difference between the average number of AFCs the difference between group 1 (26-30 years) and group 2 (31-35 years) and between group 3 (36-40 years) and group 4 (41-45 years). However, significant differences are observed between groups 1 and 3, groups 1 and 4, 2 and 3, and groups 2 and 4.

Impact on treatment outcomes: In addition to the number of eggs retrieved, the study. Can assess how age affects other key treatment outcomes such as embryo quality, implantation rate and live birth. Understanding the relationship between age and these outcomes is critical to predicting IVF success in older women. It can also guide the discussion about the likelihood of a successful pregnancy and live birth based on age-related factors. In addition, the study could examine the relationship between age and the incidence of ovarian hyper stimulation syndrome (OHSS), a possible complication of ovarian stimulation in IVF treatment, to assess the safety profile of hMG stimulation in older women.[8]

The findings suggest that while women in younger age groups (ages 26-40) have similar total egg counts, women aged 41-45 have significantly lower total egg counts. This indicates a decline in ovarian reserve and follicular response with age, especially after age 40. Clinically, these results emphasize the importance of age-based considerations in IVF treatment and counselling for those aged 40 and over.

V. CONCLUSION

The multifaceted examination of the study of total oocyte count and antral follicle count (AFC) across age groups provides a comprehensive understanding of the complex relationship between age and ovarian function in the context of artificial insemination technologies (ART). These findings significantly contribute to the existing knowledge of aging-related fertility decline and have important implications for clinical practice, patient counselling, and future research.

First, the aging-related decline in total oocyte count reinforces established understanding. Decreased ovarian reserve in older women. As women age, the pool of ovarian follicles decreases in both quantity and quality, resulting in fewer eggs during ART procedures. This decline emphasizes the importance of age-based considerations in fertility evaluation and treatment planning. Clinicians must adjust treatment protocols to account for age-related differences in ovarian response, optimizing the chances of success for patients of different age groups.

Similarly, AFC analysis further explains age-related differences in ovarian reserve, with younger women having higher follicle density than their older counterparts. AFC is a valuable marker of ovarian reserve, providing prognostic information about fertility potential and response to ovarian stimulation. The observed decrease in AFC with age highlights the progressive depletion of ovarian follicles and emphasizes the importance of early fertility evaluation in women approaching reproductive age.

By incorporating measures such as AFC and total oocyte count into treatment algorithms, clinicians can optimize treatment strategies, improve patient outcomes, and mitigate the effects of age-related fertility decline. In addition, patient counselling should emphasize the role of age in fertility and set realistic expectations for treatment results, especially for women over 30 years of age and older.

In conclusion, the study's comprehensive analysis of total oocyte count and AFC in different age groups sheds light on the complex interaction between age and ovarian function in the context of ART. By identifying and treating age-related ovarian changes, clinicians can optimize fertility treatment strategies, improve patient outcomes, and empower individuals to become parents.

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